VOLUME 77 April 1958 NUMBER 4

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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MEDICAL SECTION OF NATIONAL TUBERCU[illegible] ASSOCIATION

CONTENTS

Bronchography in Pulmonary Tuberculosis. Studies on Its Value in Patients Being Considered for Surgery. DUANE L. MERRELL AND PAUL C. SAMSON 561
The Surgical Management of Giant Cavitary Tuberculosis. JOHN W. BELL 593
Miliary Tuberculosis. A Review of Sixty-Eight Adult Patients Admitted to a Municipal General Hospital. J. PARE BIEHL 605
An Analysis of 3,485 Tuberculosis Contacts in the City of Edinburgh During 1954–1955. R. G. LOUDON, JAMES WILLIAMSON, AND JOAN M. JOHNSON 623
The Coalescent Lesion of Diatomaceous Earth Pneumoconiosis. DAVID M. CALDWELL 644
A Rapid Means of Differentiating Atypical Acid-fast Bacilli and Avian Tubercle Bacilli from Other Mycobacteria. The Neotetrazolium Inhibition Test. MERRELL GASTAMBIDE-ODIER AND D. W. SMITH 662
Differentiation of Human Tubercle Bacilli from Atypical Acid-fast Bacilli. I. Niacin Production of Human Tubercle Bacilli and Atypical Acid-fast Bacilli. KIYOSHI KONNO, RUDOLF KURTZMANN, KENNETH T. BIRD, AND ANTHONY SBARRA 669
Differentiation of Human Tubercle Bacilli from Atypical Acid-fast Bacilli. II. Clinical Application. KIYOSHI KONNO, RUDOLF KURTZMANN, KENNETH T. BIRD, AND ANTHONY SBARRA 675
Lung Density as a Measure of Tuberculous Involvement in Mice. ALFRED J. CROWLE 681
The Chemotherapeutic Activity upon Chromogenic Mycobacteria of Certain Derivatives of a Thiocarbanilide (Su 1906), Thiazoline (Su 3068), and Thiazolidinone (Su 3912). R. L. MAYER, P. C. EISMAN, T. A. GISI, AND E. A. KONOPKA 694
The Antituberculous Activities of Substituted Thiazolines and Thiazolidinones. P. C. EISMAN, E. A. KONOPKA, T. GISI, R. H. MIZZONI, AND R. L. MAYER 703
Notes:
The Chemical Constituents of *Mycobacterium Paratuberculosis*. A. B. LARSEN AND R. S. MERKAL 712
Sputum Examination Following Bronchoscopy. Its Significance in Tuberculosis. E. G. LAFORET AND J. W. STREEDER 715
Antituberculous Vaccination in the Guinea Pig with Non-Living Vaccines. DAVID W. WEISS 719
Books 725
American Trudeau Society: Notices 728
Abstracts 97

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See page 18 of advertisements

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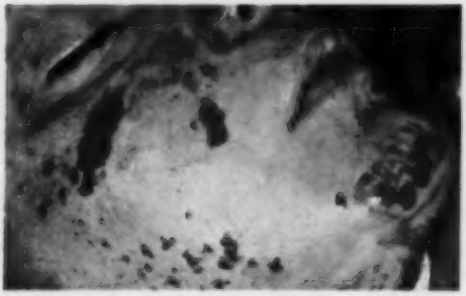

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5. *Wilkins, B.D.: Gastrointestinal Bleeding as Seen by the Proctologist, J.A.M.A. 163:1214 (April 6, 1957)*
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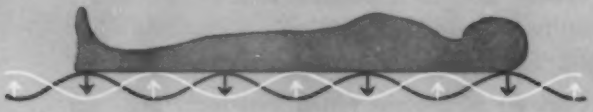

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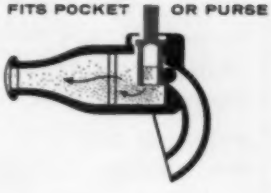

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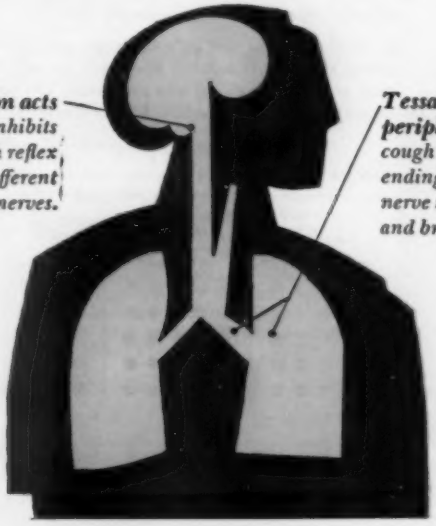
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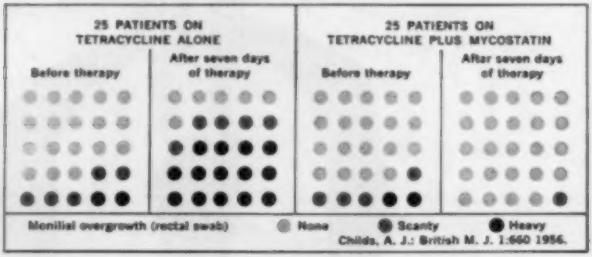

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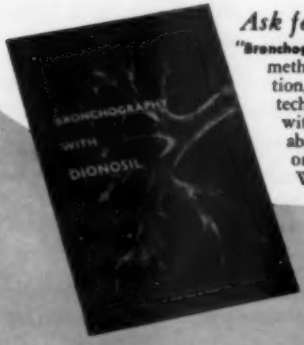

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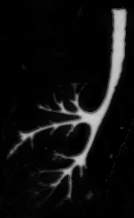

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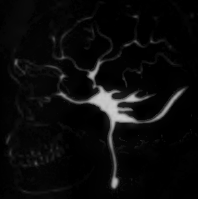

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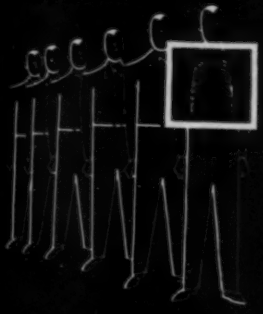

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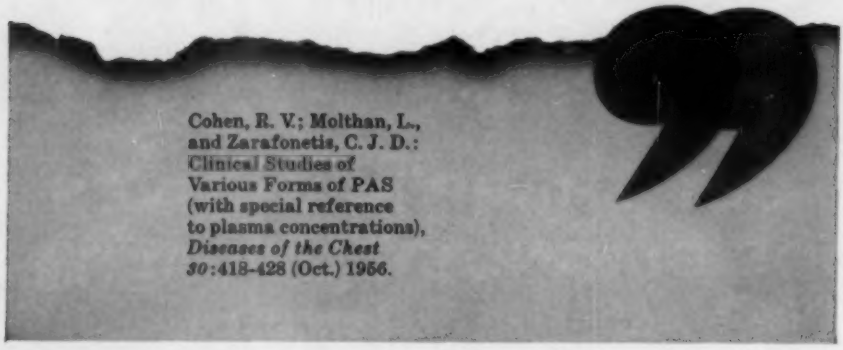

Cohen, R. V.; Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations), *Diseases of the Chest* 30:418-428 (Oct.) 1956.

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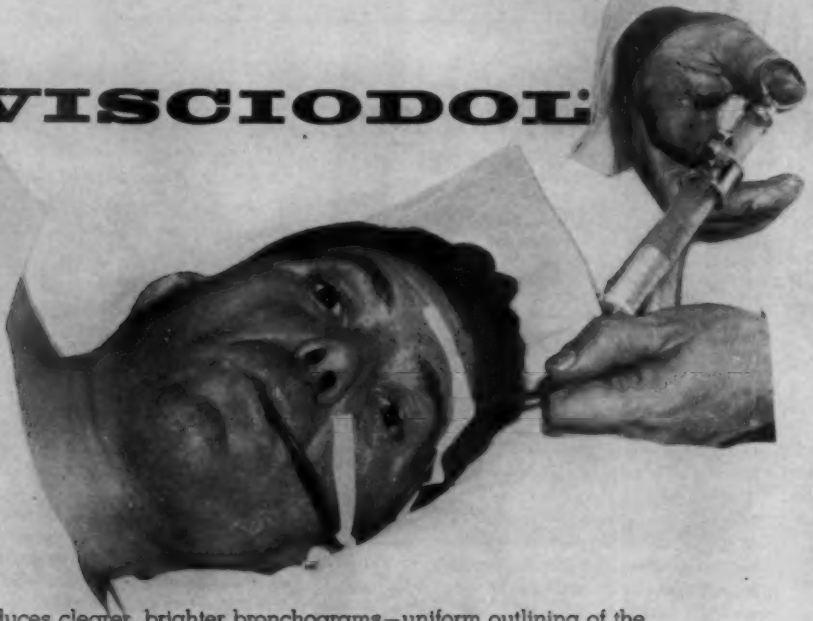

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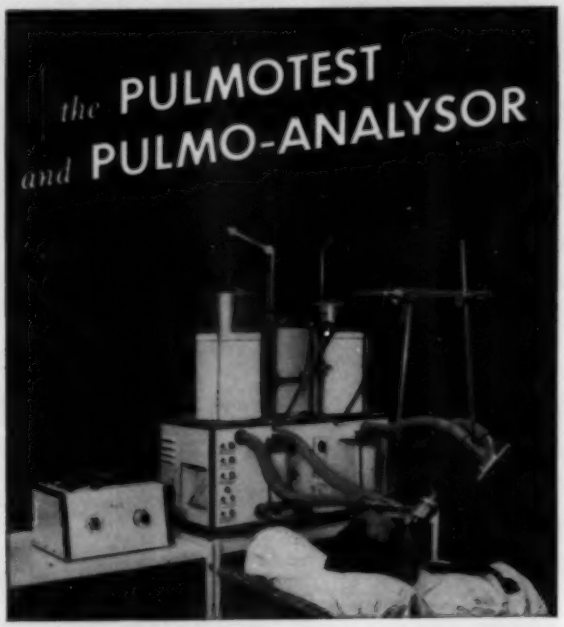

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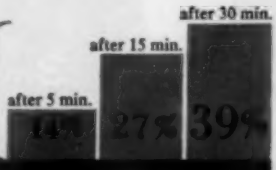

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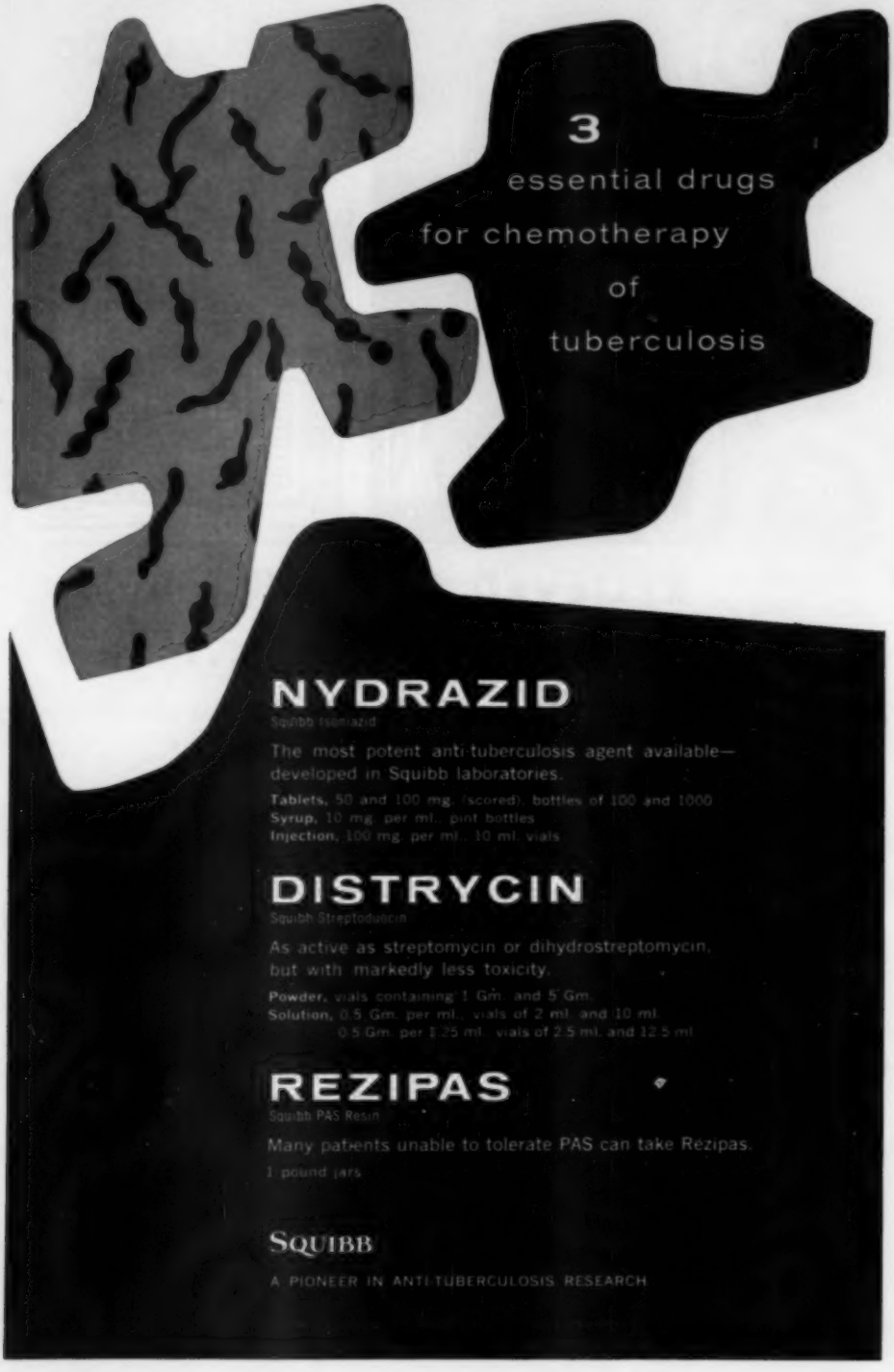
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VOLUME 77 *April, 1958* NUMBER 4

BRONCHOGRAPHY IN PULMONARY TUBERCULOSIS[1,2]

Studies on Its Value in Patients Being Considered for Surgery

DUANE L. MERRILL AND PAUL C. SAMSON

(Received for publication November 5, 1957)

INTRODUCTION

Many of the advances in the treatment of chest diseases have been in technical fields which provide information necessary for the surgical management of these disorders. New diagnostic methods and modifications of older techniques have evolved, among them the development of rapidly disappearing contrast media for bronchography. With these newer agents the general applicability of bronchography has been extended and the clinical value of the bronchogram increased. During the pre-surgical period, the use of bronchography adds much fundamental information which would otherwise remain obscure. The media in current use permit "lung mapping" of satisfactory quality and allow frequent or repeat bronchograms if necessary (1). They interfere neither with the patient's course, the interpretation of interval roentgenograms, nor with the timing of planned operations.

The diagnostic importance of contrast roentgenography of the tracheobronchial tree has long been recognized. In 1905, Jackson used bismuth oxide powder insufflated through a bronchoscope in an attempt to obtain contrast roentgenograms of the bronchi (2). This method was generally unsatisfactory. Since the introduction of Lipiodol® by Sicard and Forestier in 1922 (3), contrast oils became widely used and the basic knowledge of the bronchogram was acquired during the ensuing era.

Following these early studies, interest in bronchography rapidly increased. Much of the published work has been concerned with bronchography in tuberculosis. At the 1926 annual meeting of the American Association for Thoracic Surgery, papers by Singer (4), Tucker (5), Ballon and Ballon (6), and Archibald (7) were presented concerning this subject. The value of bronchography in the mapping of lung structure and in the demonstration of pathologic conditions of the bronchial tree was stressed.

[1] From the Thoracic Surgery Service, Highland-Alameda County Hospital, Oakland, California.

[2] California Trudeau Society Prize Essay, 1957, won by Dr. Merrill. This paper was presented at the annual meeting of the California Tuberculosis and Health Association, Los Angeles, March 28–30, 1957.

Many workers have since made evaluations of bronchography in tuberculosis, a number of which are cited (8–24). Most recent writers have emphasized that bronchograms in tuberculosis are chiefly of value in demonstrating associated bronchiectasis. The present writers believe that there are other findings of equal or greater significance. It would appear that even now the role of bronchography in determining the type and extent of surgery in pulmonary tuberculosis is not fully established, especially since the development of more efficient contrast media.

The present study is concerned with bronchography in the diagnostic evaluation of patients with pulmonary tuberculosis who are being considered for surgery. This work was undertaken to determine the value of routine bronchography in tuberculosis with the concept that it might aid in planning resective therapy. In particular, it was hoped that some light might be shed on the inter-relationship between tuberculosis and bronchiectasis, on the demonstration of elusive cavities, and on other problems which still vex the present-day thoracic physician and surgeon.

Materials and Methods

3,5-Diiodo-4-pyridone N-acetic acid (Dionosil Oily®), introduced in 1952 (25–27), has been used exclusively. There are now many reports concerning this agent. It is generally agreed that the major advantage of Dionosil over Lipiodol® is that of rapid disappearance (8, 28–32). This is due in part to its prompt absorption and excretion as an organic iodide and to the ease with which it is expectorated because of low viscosity. The degree of opacity obtained is comparable to that seen with iodized oils. One difference is that, with Dionosil, the major bronchi are shown in double contrast. Apparently it is less irritating than the water-soluble media, and not so rapidly absorbed that the time between its injection and the actual roentgenogram need be hurried (33–35).

Another advantage is that being less viscid, Dionosil Oily readily and uniformly allows visualization of the segmental bronchi and bronchioles. In the study of segmental bronchial changes, especially in pulmonary tuberculosis, this greater peripheral filling of the smaller bronchi is a distinct advantage (36, 37) and is of considerable diagnostic value. Retention of Dionosil in the lung rarely occurs, and the present writers have seen no reaction in pulmonary tissue such as was occasionally witnessed with Lipiodol (28, 38, 39).

The optimal time interval between bronchography and operation has not been definitely established for Dionosil, but it appears from this study and from the work of others that a waiting period is unnecessary (32). Nevertheless, the practice was usually followed of allowing at least one week between the bronchogram and elective surgery. In several instances, however, surgery has been performed the day following bronchography with no apparent ill effect. Bronchograms have been repeated within one day when necessary because of inadequate filling or faulty technique.

Some workers prefer aqueous Dionosil (40), and others believe a suspension of sulfanilamide and iodized oil to be the best agent (41). The present writers have had no experience with these media. An excellent review of the relative usefulness of various agents has been presented by Domm and associates (42).

Procedure

Following premedication with Demerol®, Sodium Nembutal®, and atropine, the pharynx and nares are anesthetized with 2 per cent Xylocaine®. This is done by repeated spraying on inspiration followed by encouraged cough and expectoration. After a few seconds a blunt-tipped catheter is then introduced through the nose into the designated bronchus

under fluoroscopy. When combined with bronchoscopy, the catheter is inserted in the same manner immediately upon removal of the bronchoscope. These procedures are conducted as rapidly as possible to retain the maximal suppression of the cough reflex. If, at the time the catheter is introduced, inadequate anesthesia exists, a few milliliters of Xylocaine are injected through the catheter and coughing is again encouraged.

Two patients developed bronchorrhea following this type of anesthesia. In both, the study was abandoned at the time and later completed successfully with larger doses of atropine. No other reactions which could be attributed to the anesthetic were observed.

One patient was too depressed following premedication to cooperate and the procedure was performed at a later date. Cough has not been a major problem in the cooperative patient.

Filling of the bronchial tree is accomplished by a method which was developed independently, but which closely resembles the technique described by Priviteri (1) as "physiologic bronchography."

The physical properties of the agent used allow its distribution throughout the bronchial tree by deliberate inhalation. Specifically, under fluoroscopic guidance, 15 to 20 ml. of Dionosil Oily are injected rapidly through the catheter into the designated bronchus as a pool or bolus. At the immediate completion of the injection the patient is instructed to inhale deeply. This disperses the Dionosil along the walls of the bronchi. During this maneuver the patient lies supine and horizontal on the table. Any nonvisualized segments are then filled by selective positioning and controlled inhalation until satisfactory filling of the unilateral bronchial tree is ascertained (1, 43–45).

Several spot films are taken during this procedure. With the first side satisfactorily filled under fluoroscopic control, large films are taken in frontal, oblique, and *lateral* projections. When bilateral studies are desired, a second similar injection is made into the opposite bronchus. It is seldom necessary to use more than 10 ml. to complete the filling of the second side. Roentgenograms are then taken in the postero-anterior and oblique positions only. The patient is then placed prone on the table, which is tilted head downward, and is encouraged to cough and expectorate the media. Post-tussive and delayed films are taken as indicated. Upon return to the ward, hourly postural drainage is ordered for five hours and aerosol inhalations of Alevaire® and Isuprel® are given.

Bronchography in children is performed under light general endotracheal anesthesia supplemented with a topical agent such as Xylocaine or cocaine. Under strict fluoroscopic guidance, limited volumes of the media (5 to 10 ml.) are injected by means of a small plastic catheter previously passed through the lumen of the indwelling endotracheal tube; thus laryngospasm and anoxia are prevented. Unilateral studies are done, following which as much of the agent as possible is aspirated with the catheter. Under good anesthetic control, bronchograms can be made on the second side as a leisurely procedure. It is advisable to observe the child's respirations carefully throughout the examination.

Contraindications and Complications.

Certain contraindications to bronchography were observed. When active bronchitis was present, appropriate aerosol and antimicrobial therapy was instituted for a week and improvement was confirmed bronchoscopically prior to bronchograms. Patients with acute exudative tuberculosis or recent bronchogenic spread were not subjected to bronchography.

Two minor complications developed. One patient, initially thought to have unresolved pneumonia, was later proved to have acute tuberculosis. Following bronchography, a rapidly developing fever of 104° F. slowly declined on aerosol therapy. The other patient with a cavitary, atelectatic middle lobe developed temporary mild fever following the procedure.

Study Material

The present study is limited to bronchography in tuberculosis. Many significant facts concerning bronchograms in nontuberculous conditions have emerged, however; therefore

the over-all statistics concerning bronchography on the Highland-Alameda County Hospital Thoracic Surgery Service during the year 1956 will be given as background. The results of bronchography in disease other than tuberculosis will be presented in a subsequent paper.

During this period, 131 bronchograms were performed on 105 patients. These cases comprised 57.6 per cent of the major operative admissions and 24.6 per cent of the nonoperative admissions to the service. Ordinarily, bronchography has not been performed in certain types of cases, such as in the early management of chest injuries or in solitary nodules of the lung. Moreover, patients with carcinoma of the lung proved by examination of bi-

TABLE 1

DIAGNOSIS OF CASES STUDIED BY BRONCHOGRAPHY

Diagnoses			Number of Cases
Tuberculosis (proved)			38
No previous surgery		29	
Parenchymal disease	24		
Empyema	2		
Mediastinal abscess	1		
Lymph node involvement with bronchial obstruction	2		
Previous surgery		9	
Thoracoplasty	2		
Resection and thoracoplasty	2		
Parenchymal resection	3		
Pneumonectomy	1		
Decortication	1		
Tuberculosis (possible but unproved)			5
Bronchiectasis without evidence of parenchymal disease	3		
Solitary nodule of lung	2		
Total			43
Nontuberculous diseases			48
Bronchiectasis	9		
Lung abscess, cysts, and residual pseudo-cyst	5		
Carcinoma	5		
Bronchostenosis (congenital or etiology undetermined)	6		
Fibrous pneumonitis	2		
Tracheal obstruction	2		
Carcinoma	1		
Mediastinal thyroid adenoma	1		
Traumatic hemothorax	1		
Mitral stenosis	1		
Emphysema	1		
Chondrosarcoma, rib	1		
Empyema, chronic	1		
Miscellaneous			14
Obesity	1		
Congenital anomaly, tracheobronchial tree	1		
Others, undiagnosed, no disease, normal bronchogram	12		
Total			62
Total (all cases)			105

TABLE 2

BRONCHOGRAMS REPEATED FOR OBSERVATION OF THE PATIENT'S COURSE

Diagnoses and Procedure	Number of Repeat Bronchograms
Tuberculosis (proved)	10
Empyema (decortication)	1
Contracted, cavitary right upper lobe (lobectomy and thoracoplasty)	2
Occult fistula-old (decortication and revision of bronchus)	3
Occult fistula-old (thoracoplasty)	2
Advanced upper lobe disease with major bronchial distortion (thoracoplasty)	2
Tuberculosis (possible but unproved)	1
Bronchiectasis with hemoptysis (conservative therapy)	1
Nontuberculous diseases	4
Traumatic hemothorax (decortication)	1
Traumatic hemothorax (thoracentesis)	1
Coccidioidomycosis (lingulectomy)	1
Bronchiectasis (aerosol and postural drainage)	1

opsy material or strongly suspected on routine roentgenographic examination usually have not had bronchograms.

Whenever indicated, bronchograms were repeated. Of the 131 bronchograms performed, 26 were repeat studies: 15 for observation of the patient's course; 9 because of inadequate filling (6.8 per cent); 2 because of faulty X-ray technique. Priviteri found incomplete filling in 7 per cent of his one hundred and seventy cases. This compares closely with the findings in the present study.

A diagnostic tabulation of the entire 105 patients who were studied by bronchography during the past year is given in table 1. The material analyzed below concerns the first 43 patients, those with known or suspected tuberculosis.

The 15 bronchograms which were repeated for observation are summarized in table 2. A single repeat study was made on each of 10 patients: Eight were postoperative; one was on a patient with a mild basilar bronchiectasis after conservative therapy; and the tenth was performed on a patient with recurrent hemoptysis, eight months following the first examination. Two repeat studies were made on a patient between the operations of a staged procedure. Three repeat bronchograms were done on one patient following the initial examination which demonstrated a bronchial stump sinus (*see* figure 12).

Thoracotomy and resection in patients with tuberculosis have brought about an increasing awareness of the limitations of the standard roentgenogram. Surgical findings and pathologic examinations have contributed to diagnostic accuracy. These studies have revealed changes in the segmental bronchi comparable with those in the major bronchi which became better understood as the role of bronchoscopy in pulmonary tuberculosis became established (46–48). Janes (49) noted the increased awareness of atelectasis as indicating bronchial disease with occlusion; he likewise stressed that an unexpected finding of tubercle bacilli in the sputum might well be due to intrinsic bronchial disease. Some writers currently indicate that in most cases preoperative roentgenograms alone may be relied upon to identify cavities and areas of disease (50). Others have found, however, that the correlation between roentgenographic shadows and the pathologic findings in resected specimens was only good in slightly more than half, and fair or poor in the remaining instances (51). It has also been noted

that some significant pathologic conditions may produce no roentgenographic change (52).

Bronchography with Dionosil Oily permits the graphic demonstration of segmental bronchial changes preoperatively. A fairly broad personal sur-

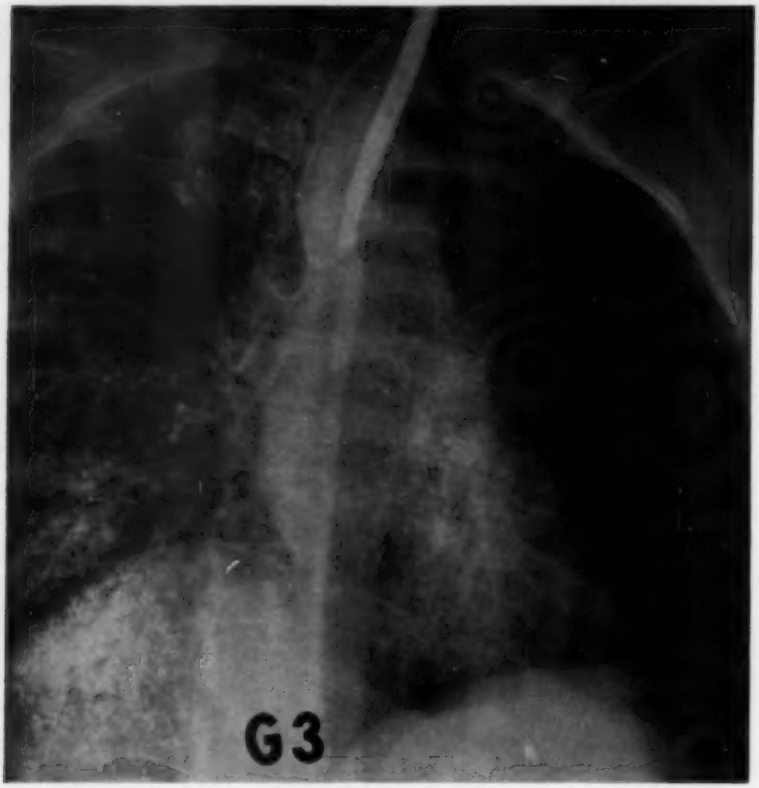

FIG. 1. Bilateral bronchogram with Dionosil Oily demonstrating many of the pathologic findings in pulmonary tuberculosis which may be seen on bronchography.

On the right, there is contraction and upward retraction of the upper lobe with deviation of the trachea to the right. All of the segmental bronchi of the upper lobe are markedly bronchiectatic and end blindly with no distal filling of the smaller bronchioles. A large spherical cavity is demonstrated beyond the zone of terminal segmental stenosis. There is compensatory upward retraction and slight overexpansion of the middle lobe. Associated bronchiectasis of one division of the superior segment of the lower lobe may be noted. The diaphragm is elevated.

On the left, a partial induced pneumothorax is present. There is mild ectasia with terminal blunting of the apical posterior segment of the upper lobe with no filling of the distal smaller bronchioles. Two branches of the anterior segment appear slightly dilated and terminate blindly at their junction with thick-walled, "blocked" cavities.

There is a suggestion of irregularity and dilatation of one or two of the basilar segmental bronchi on each side.

gical-pathologic experience indicates that certain types of bronchial disease demonstrable on the bronchogram are highly suggestive of distal parenchymal pathology (figure 1).

Pulmonary Tuberculosis Without Previous Surgery

The following brief review of pertinent concepts of the pathology, chemotherapy, and bacteriology in tuberculosis may help to correlate bronchographic with pathologic findings.

Bronchial involvement in surgically resected specimens is now recognized as a frequent finding. In a study of six hundred and fifty-eight consecutive surgical specimens, Olsen and co-workers found endobronchial disease in 92 per cent (53). These were classified as chronic bronchitis, 29 per cent; tuberculous bronchitis, 20 per cent; bronchiectasis without endobronchial tuberculosis, 21 per cent; and bronchiectasis with endobronchial tuberculosis, 20 per cent. Interstitial fibrosis, tuberculous endobronchitis, and extraluminal compression of a major bronchus were factors which favored residual bronchiectasis. Other workers have found a frequent association between tuberculosis, bronchiectasis, and tuberculous endobronchitis (40 to 80 per cent), depending in part upon the criteria used (54–58).

With regard to the mechanics of healing in the pre-drug era, Corylloss (59) emphasized in 1933 that occlusion of the draining bronchus was an essential factor in cavity closure and healing. In 1940, Auerbach and Green (60) studied cavity healing and concluded that inspissation of caseous material was the most common mechanism of cavity closure. In all instances of closed cavities, however, they found that the draining bronchus ended blindly in an area of fibrosis at the level of the cavity wall.

There are differences in the pathology after prolonged chemotherapy and, as described by Auerbach (61), these consist of thinner capsules around necrotic foci, thinner cavity walls, and a decrease in pulmonary fibrosis. He states that cavity closure occurs with a patent and re-epithelized draining bronchus and that open-cavity healing occurs with greater frequency. Thompson (62) found, however, that open-cavity healing is still unusual (9.6 per cent of 240 resected cavitary lesions) and that the pathologic changes are otherwise quite similar to those observed in the pre-drug era.

In view of the fact that open-cavity healing does occur, it is thought by some workers that this ideal potential of drug therapy is not realized frequently enough and that several factors may be responsible. Russell and associates (63) state:

> Failure of caseous material to soften may occur. Premature closure of the bronchus may hamper elimination. . . . Delay in mechanical elimination may continuously block one of the drugs from activity within the cavity and so allow organisms resistant to the other to be selected. Irreversible fibrotic pericavitary lesions might interfere with the mechanics of emptying.

Pagel and Simmonds (64) point out that an open cavity may be conversion of the cavity into a space with a patent bronchus and residual caseous material in one or more recesses.

Bacteriologic studies of resected specimens have been informative (65). Hobby and associates believe that surviving acid-fast bacilli in a suppressed metabolic state are present in residual lesions and that such a state is reversible (66, 67). This work has not been confirmed. It is known, however, that a definite correlation exists between the recent discharge of tubercle bacilli in the sputum and the likelihood of finding culturable bacilli in the resected tissues. It is also believed that sparse but viable bacilli in certain old closed lesions are capable of reactivating disease (68).

Persistence of bacilli which have become resistant to available drugs seriously increases the incidence of surgical complications associated with limited (segmental or wedge) resections. In such cases Bell (69) recommends lobectomy rather than segmental resection, thus fitting the surgery to the anticipated bacteriologic findings in the lesions.

Gordon and associates (16) have shown that the bronchograms observed in resected specimens injected with radiopaque material compare closely with those in living patients. Bronchography is of proved value in the demonstration of bronchiectasis associated with tuberculosis. Buckles and associates (14) found that bronchiectasis was diagnosed bronchographically in 73.2 per cent of their resection cases but confirmed by postoperative microscopic study in only 53 per cent.

Corpe and Hwa (70) made a correlated bronchographic and histopathologic study of bronchial disease in two hundred and sixteen tuberculous patients, using iodized oil. In 80 per cent of the specimens which were interpreted as showing bronchiectasis on the bronchogram, this was confirmed pathologically. It is of special interest that there was demonstrable associated bronchiectasis in forty-six patients in their series who had no pulmonary resection. Thirty-six of these were followed for an average of nineteen months. During this period, no patient showed profuse or foul expectoration. One patient had a single hemoptysis. Eighty-nine per cent had stationary roentgenographic findings after discharge; 11 per cent showed evidence of improvement after discharge; none showed roentgenographic evidence of progression.

The bronchographic pattern of bronchiectasis in tuberculosis differs from that seen in pyogenic bronchiectasis (71, 72). Any segment or combination of segments may be involved in either condition. Both conditions may be present and demonstrable in the same patient. Frequently, however, bronchiectasis associated with tuberculosis occurs in the apical and superior segments, areas most frequently involved by parenchymal lesions at some stage in the disease. As is well known, pyogenic bronchiectasis usually involves the dependent portions of the lung. In reviewing Olsen's work (53) it is apparent that bronchiectasis in tuberculous specimens is associated with endobronchial tuberculosis in approximately half of the specimens showing ectasia. It is the impression of the present writers that bronchiectasis in tuberculosis can be related to endobronchial disease in some instances and to parenchymal fibrosis (or healing) in others. Bronchial occlusion with ectasia, however, implies bronchial or bronchocavitary tuberculosis. This is an important consideration which will be discussed in greater detail below.

Bronchographic and Pathologic Observations in the Present Study

Twenty-four of the 38 patients with tuberculosis studied by bronchography in the present series had pulmonary parenchymal involvement and had not had previous surgery. These patients were referred for operation on recommendation of the staff after acceptable periods of sanatorium care, antituberculous therapy, frequently pneumoperitoneum (73), and supportive adjuvants. Such patients usually demonstrated extensively damaged parenchyma with actual destruction, contraction, cavitation, or persistent fibrocaseous disease.

Gordon's observations have been repeatedly confirmed. In advanced disease, there are typical bronchographic findings of dilatation, crowding together, and

irregularity in the segmental bronchi in areas of parenchymal disease; these bronchi end rather abruptly so there is no filling in the distal bronchial tree. This probably represents the effect of fibrotic replacement of normal lung tissue plus atelectasis. The anatomic delineation of the segmental bronchi by bronchography aids in identifying contracted areas of lung and in recognizing bronchial distortion which may be apparent in no other way. The cases in this series, with a comparison of the bronchograms and the resected specimens, are summarized in table 3.

The possible courses of cavity healing have been reviewed. Cavitary or fibrocaseous disease not responding to antituberculous treatment is generally accepted as an indication for surgery, usually resection (74). The demonstration of cavities by bronchographic filling is sporadic even with modern chemotherapy and the radiopaque agents now in use (figure 2). When a cavity is outlined the question arises whether open healing has occurred (figure 3); the implication may be that it has. This is a significant question which requires further investigation.

Bronchiectasis with Terminal Segmental Stenosis (Terminal Block)

The question arises whether bronchography aids in the identification of cavitary or fibrocaseous disease which otherwise might not be interpreted as such on routine roentgenographic examination or planigraphy. If the draining bronchus does not remain patent but becomes stenosed, the area previously drained may contain large abscessed cavities filled with liquid or inspissated necrotic material. On routine roentgenographic examination and on planigrams, these areas may show as diffusely opacified zones, often interpreted as atelectatic lung or as fibrosis, with the implication that healing has occurred. This is particularly true if the area represents an original cavitation which has been followed by serial roentgenograms. The segmental bronchial changes seen on bronchography may be the only indication of the extensive parenchymal disease remaining. The abrupt termination of ectatic segmental bronchi so involved is usually seen in the upper lobes (figure 4). On gross pathologic examination, this represents terminal bronchostenosis of the segmental or subsegmental bronchi; beyond, there may be fibrosis, atelectasis, fibrocaseous disease, or cavities of variable size. Cavities as large as 5 cm. in diameter have been found distal to such stenotic segmental bronchi after resection when they were indiscernible by roentgenogram, including planigraphy (figure 5). In the experience of the present writers, bronchograms which reveal these characteristic findings of advanced disease have aided materially in deciding that surgery should be done.

Especially in cases in which tubercle bacilli occasionally appear in the sputum, it remains a question whether this type of bronchial involvement with distal parenchymal change represents a healed lesion or a static one. While these areas may appear as possibly healed lesions on plain films, they may actually represent blocked cavities and residual necrotic or fibrocaseous disease (figure 6). If residual disease should be resected earlier in view of possible reactivation, and the emergence of drug-resistant strains of tubercle bacilli, bronchograms showing the above-described changes are diagnostic. Thurn's concept that periodic bron-

TABLE 3

COMPARISON OF BRONCHOGRAMS AND RESECTED SPECIMENS

(24 Cases of Parenchymal Disease—15 Resections)*

Patient	Type of Resection	Bronchogram		Resected Specimen		
		Bronchiectasis	Terminal Block	Bronchiectasis	Terminal Block	Type of Disease Distal to Block
M. A. (Fig.3) (age 7)	RUL	+-0	0 (Cavity)	+-0	0	"Open healing" multicavitary
E. B. (Fig. 5)	AP segment of LUL	+	+	+	+	Fibrocaseous with 5 cm. cavity
R. P.	AP segment of LUL, wedge segment LLL	+ +	0 0	+ +	0 0	Healed, fibrosis, no active tuberculosis
H. L.	RUL	+	+ (Cavity)	+	+	Fibrocaseous with 3 cm. cavity
C. J. (Fig. 2)	RUL	+	+(Cavity)	+	+	Fibrocaseous with 4 cm. cavity
P. R.	AP and A segment of LUL, S segment of LLL	+ +	+ (Cavity) +	+ +	+ +	Fibrocaseous with multicavities; fibrocaseous with 8 cm. cavity
J. B.	AP segment of LUL	Inadequate bronchograms × 2		+	+	Caseation necrosis with 3 cm. cavity
C. G. 1 infectious sputum	LUL	+	0	+	0	Silico-coccidioidomycosis; multiterminal cavities

C. S.	AP segment of LUL	+	+	+	+	Fibrocaseous with 3 cm. cavity
M. V.	LUL and apical subsegment of LLL; S segment	+ +	+ +	+ +	+ +	Fibrocaseous Fibrocaseous
Z. J.	Apical segment RUL	+–0	+–0	+–0	0	Fibrocaseous, 2 cm. terminal cavity
W. H. (Fig. 4)	P segment of RUL, wedge of RML	+	+	+	+	Fibrocaseous, 2.5 cm. cavity; 1.5 cm. nodule in RML
E. J. (Fig. 7)	Sub-superior segment of LLL	+ plus + in lingula	+ 0 in lingula	+ Lingula preserved	+	Fibrocaseous
B. S. (Fig. 8)	L. lung	+	+	+	+	Fibrocaseous—destroyed lung
E. D.	R. lung	Massive cavity		Massive cavity		Destroyed lung

* Fifteen patients had fifteen resections. Five patients had thoracoplasty. Four patients were nonoperative cases.

KEY: + present 0 absent +–0 questionable

Lobes
RUL Right upper lobe
RML Right middle lobe
RLL Right lower lobe
LUL Left upper lobe
LLL Left lower lobe

Segments
AP Apical-posterior
A Anterior
P Posterior
S Superior

Subsegments
Not abbreviated

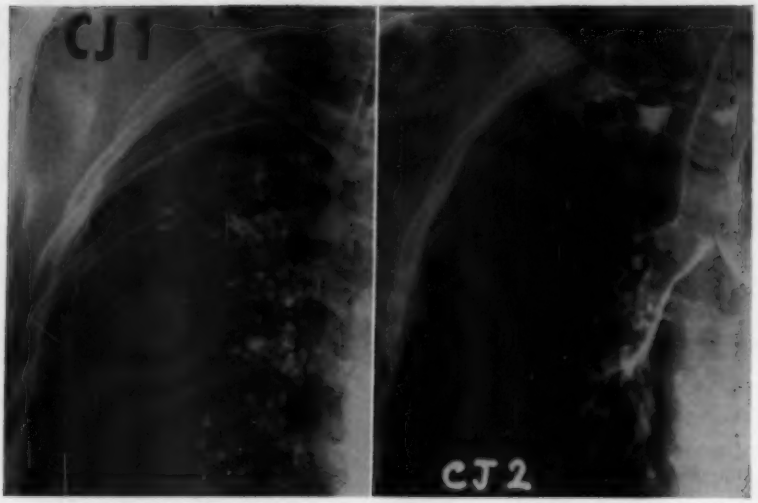

FIG. 2. The demonstration of cavities by bronchography is sporadic. These bronchograms were made six weeks apart. CJ1 shows upward angulation of the right upper lobe bronchus with bronchiectasis and segmental terminal stenosis of the apical and posterior segments. No cavity is demonstrated. CJ2, six weeks later, shows that a large cystic cavity is partially filled.

A right upper lobectomy was performed. Fibrocaseous disease with active granulation at the bronchocavitary junction was found.

chography would identify segmental bronchostenosis relatively early is interesting (20). Bronchography may play an important role in determining whether or not such residual lesions should be resected. Certainly terminal segmental bronchial stenosis cannot be demonstrated by any other technique.

Bronchiectasis Without Terminal Segmental Stenosis

Bronchiectasis associated with tuberculosis is now apparently accepted as a clear indication for resection (24, 75). The relatively low incidence of active tuberculous endobronchitis, associated with bronchiectasis, demonstrated by Buckles, Olsen, Corpe, and others and the remarkably uncomplicated course of the unoperated cases followed by Corpe and Hwa make one wonder whether all bronchiectasis is an indication for resection. Even in 1926, Singer (4) emphasized that pathology demonstrable by bronchography which is associated with a concurring clinical picture requires no discussion, but that bronchograms must be read with experience and with a consideration of clinical factors. Fluoroscopic guidance is of great importance as Phillips has stressed. The present writers have often found that what appears to be marked dilatation of the bronchi seen during inspiration and on the initial spot films is no longer apparent on the well-filled but static bronchograms. A careful evaluation of numerous films taken during the procedure is necessary.

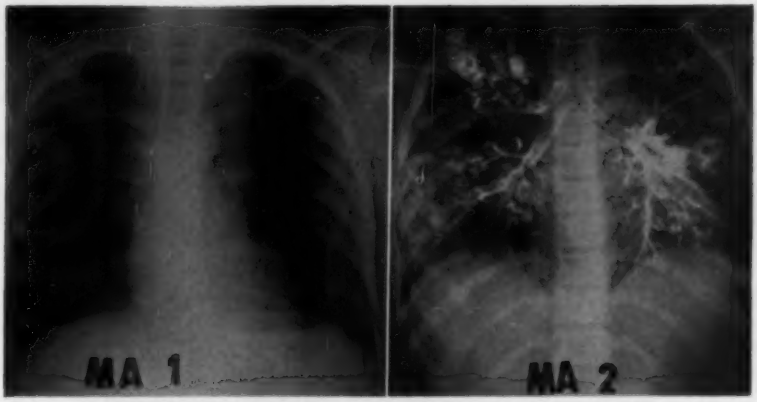

FIG. 3. *"Open healing":* The patient, a 7-year-old child, had been on chemotherapy for proved tuberculosis for one year. The bronchogram shows multicavitary right upper lobe.

A right upper lobectomy was performed. On pathologic examination, the cavities were epithelized with no active granulation tissue, but some surrounding fibrosis.

FIG. 4. Bronchogram showing mild ectasia and terminal stenosis of the posterior segment of the right upper lobe. This bronchus terminates at a zone of opacification where serial films previously showed a cavity. In this case the terminal segmental stenosis argued strongly for resection. Other areas of bronchiectasis are demonstrated in the apical and anterior segments and in the proximal middle lobe.

Segmental resection of the posterior segment and wedge resection of a nodule in the middle lobe were performed. The posterior segmental lesion was a solidly inspissated cavity containing culturable bacilli. Organisms were susceptible to the major chemotherapeutic agents.

FIG. 5. This patient had a known cavity in the left apex which had apparently closed over a two-year period. Sputum remained positive for *M. tuberculosis* on culture, but surgery was refused. Note the lack of convincing evidence of cavity on regular films (EB 1 and 2). The bronchogram reveals bronchiectasis with terminal segmental stenosis involving the apical posterior segment of the left upper lobe (EB3).

Resection of this segment was performed. A 5 cm. necrotic cavity filled with thick liquid material was found on pathologic examination. The patient remains negative twenty months following surgery.

It has been the writers' experience that many cases of tuberculosis show adjacent bronchiectasis in segments which are not destroyed and are only minimally involved with fibronodular disease (figure 7). In general, the incidence of active endobronchial disease in these segments is low. It is not clear whether bronchiectasis of this sort should be resected. By the same token, there might be some danger in resecting areas of advanced parenchymal disease and not

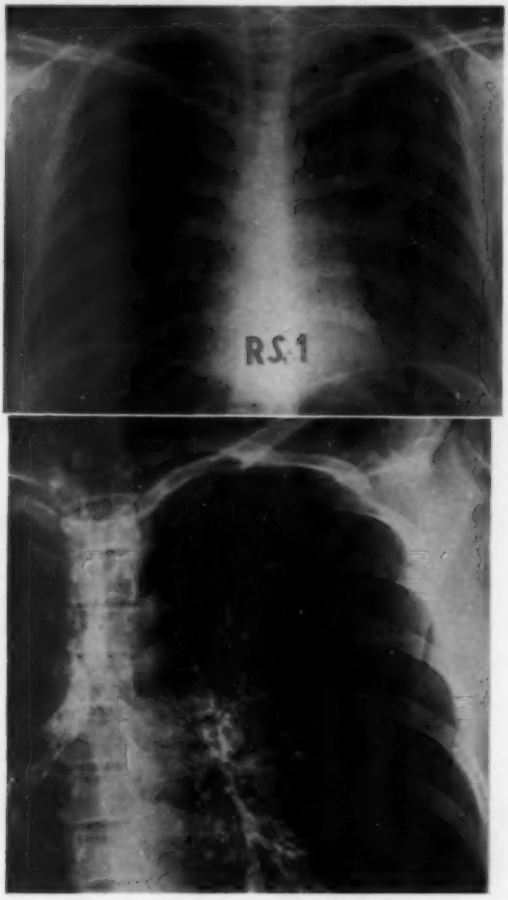

FIG. 6. Postero-anterior roentgenograms show persistent disease at the left apex. Bilateral bronchogram is normal except for slight ectasia and terminal segmental stenosis of the apical posterior segment of the left upper lobe with lack of filling of the smaller bronchioles.

This segment was resected and fibrocaseous disease with a cavity measuring 2 cm. in diameter was found.

removing adjacent functional lung tissue which shows unobstructed bronchial ectasia on the bronchogram.

In tuberculosis resection, the palpation of various segments of the lung at the time of surgery remains one of the best indications whether or not resection of the area should be done. Where there is functioning, aerating lung with fibronodular disease, there may be bronchographically demonstrable bronchiectasis without terminal segmental bronchostenosis. The present writers do not believe that this finding per se is necessarily an indication for resection, and at the present time are not removing such segments. An evaluation of the advisability of this concept should be feasible with postoperative bronchograms in resection cases in which this policy has been followed.

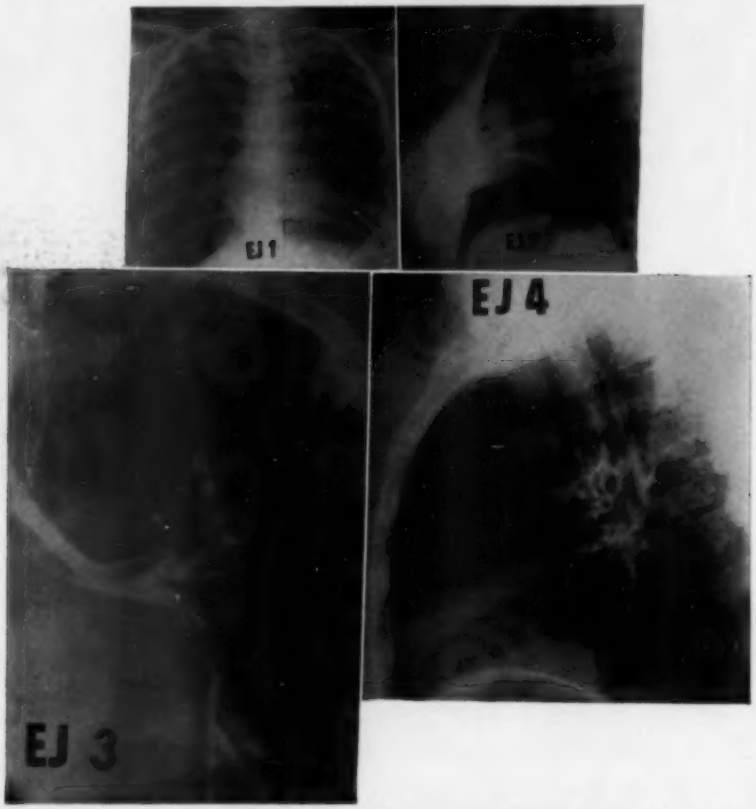

FIG. 7. Postero-anterior and left lateral roentgenograms. Bronchogram showing bronchiectasis involving the lingula without bronchostenosis. There is ectasia with terminal segmental stenosis of a subsuperior segment seen best on the lateral bronchogram.

A subsuperior segmental resection was performed. No other pathologic disease was present. The resected subsegment showed fibrocaseous tuberculosis.

Here again the presence or absence of symptoms due to the bronchiectasis itself must be duly considered. Thus, persistent purulent sputum demonstrated bronchoscopically to be coming from the ectatic lobe or segment would argue for total rather than segmental removal.

Major Bronchial Distortion in Advanced Disease

When combined with bronchoscopy, a study of the bronchogram is of value in the demonstration of bronchostenosis involving the major bronchi and the proximal portions of the segmental bronchi. The extent of such stenotic lesions may not be directly visible but is demonstrable by contrast roentgenography (*See* figure 15).

In severely contracted left upper lobes with marked cephalad angulation of the left main bronchus, the distortion may preclude an adequate bronchoscopic evaluation in areas of contemplated resection. Preliminary thoracoplasty may be indicated in such cases; by seriatim bronchography, it has been possible to demonstrate lessening of the bronchial angulation and improvement in the drainage from such areas (figure 8). In far advanced bilateral disease with similar bronchial distortion, severe cough, and poor sputum drainage related to architectural abnormalities, bronchography has aided in the establishment of such a diagnosis; and repeat studies have revealed the improved bronchial pattern after thoracoplasty, with palliation of the patient's major symptoms.

Tuberculous Lymphadenopathy with Mediastinal and/or Bronchial Involvement

Since Brock (76, 77) demonstrated obstructive stenosis of the right middle lobe bronchus due to lymphadenitis, there has been an awareness of the effect of pathologic lymph nodes upon adjacent bronchi, particularly in childhood tuberculosis. The concept of the "middle lobe syndrome" was further advanced by Graham, Burford, and Mayer (78) in 1948. Lymph node disease, although most often tuberculous, may be any sort of granulomatous infection or may be due to nonspecific hyperplasia. It is frequently amenable to surgery; in children particularly, large masses of lymph nodes should be removed in some instances, as the present writers have previously shown (79).

Williams and Anderson (80) studied forty patients with primary tuberculosis in infancy and childhood; in thirty-two, bronchiectasis developed; and in ten, permanent bronchostenosis (figure 9). They believed that tuberculous infection of the involved segment often played a contributory role, whereas Brock's original concept was that the disease was primarily lymphatic. The two must be related, obstruction contributing to poor drainage which in turn contributes to lymphadenitis.

Vojtek (81) has recently stressed the importance of bronchography before and after excisional surgery in children. Areas of atelectasis in children with primary tuberculosis frequently show total segmental bronchial obstructions; at surgery there is significant lymphadenopathy (often caseous), bronchostenosis, and total pneumonic involvement of the lobe or segment. Localization and confirmation of the bronchial component with its pathophysiologic complications is readily demonstrable with an adequate bronchogram. Mediastinal lymphadenopathy

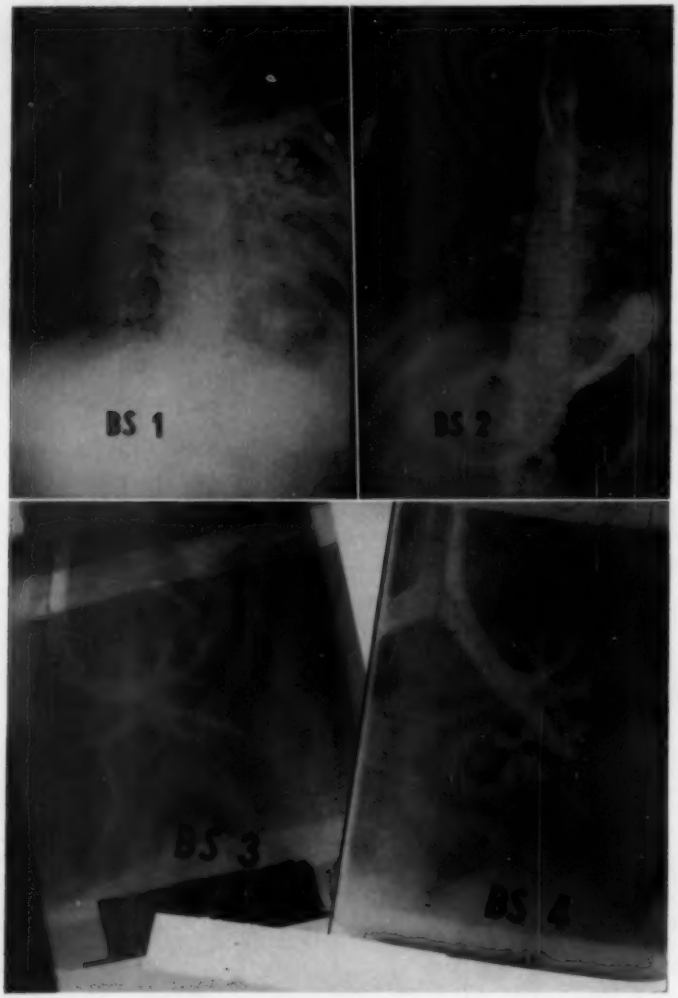

FIG. 8. The effect of preliminary thoracoplasty on major bronchial distortion.

This patient had extensive tuberculous involvement of the left lung with upward angulation of the left main bronchus which impaired drainage and prevented bronchoscopic evaluation (BS1, BS3).

Following seven-rib thoracoplasty there was a return of the bronchus to a normal position with marked improvement in cough and diminution of sputum (BS2, BS4).

Bronchoscopic evaluation was then possible and, when no evidence of active bronchial disease was seen, an uneventful pneumonectomy was performed.

The patient's sputum was negative for *M. tuberculosis* and she was discharged asymptomatic one year following surgery.

FIG. 9. A 15-year-old patient had known childhood tuberculosis. She entered the hospital with recurrent hemoptysis of one year's duration. On bronchoscopy the site of bleeding was the right middle lobe. Bronchography shows a calcified lymph node adjacent to the middle lobe bronchus with bronchiectasis of the lateral segment.

A right middle lobectomy was performed, with relief of the patient's symptoms. There was calcification of the lymph node which was eroding the bronchus.

without bronchial obstruction may also be demonstrated bronchographically as marked widening of the carina of the major bronchi (figure 10).

In one patient with a tuberculous "cold abscess" lying in the anterior mediastinum, bronchography showed no bronchial or pulmonary abnormalities, but aided in the diagnostic exclusion of a bronchopleural communication.

Tuberculous Empyema and "Captive Lung"

Bronchography has been used in the past to aid in the decision whether or not an unexpandable lung under pneumothorax or empyema was healthy enough to

FIG. 10. Bronchographic study of childhood tuberculosis. A 4-year-old child had sputum positive for *M. tuberculosis* for a six-month period. On intensive chemotherapy, there was persistent disease without clearing of the roentgenographic abnormalities. Bronchoscopy revealed purulent sputum emerging from the right middle lobe and the medial basal segment of the right lower lobe, with marked widening of the carina. The bronchograms show obstruction of this lobe and segment with widening of the carina compatible with mediastinal lymphadenitis.

At surgery, the middle lobe and the medial basal segment of the lower lobe were removed. They were atelectatic and purulent on section. Large caseous lymph nodes adjacent to each of these bronchi contributed to the obstruction. The nodes were removed as widely as possible.

make decortication advisable (16). With revival of interest in decortication (82), the introduction of new techniques, and their application to the pleural complications of tuberculosis, the anatomic and physiologic condition of the underlying lung has become of great importance (83).

Two patients in the present series underwent decortication for tuberculous empyema. Preoperative bronchograms in both patients revealed widening of the

bronchi in the involved lung, lack of distal tapering with no filling of the small bronchioles, and clumping; all signs indistinguishable from bronchiectasis except on clinical grounds. Following decortication, bronchograms were performed showing restoration of the bronchial tree to normal (figure 11). This condition represents "pseudo-bronchiectasis," a condition of the bronchial system which is

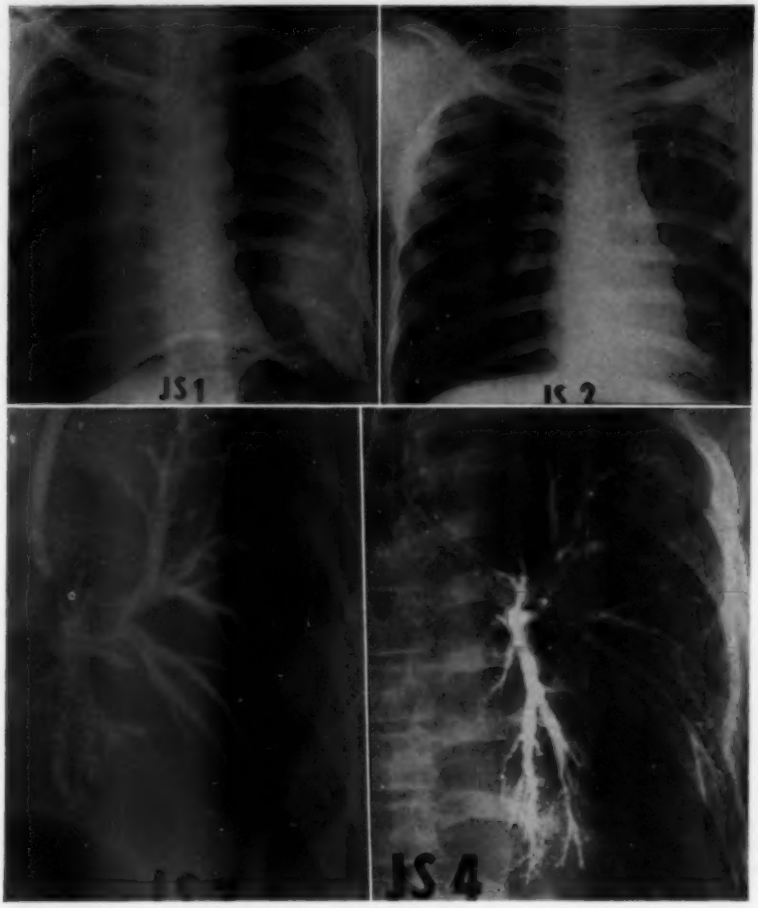

FIG. 11. The effect of decortication on compressed lung as demonstrated by bronchography:

JS1: Tuberculous empyema following induced pneumothorax.

JS2: Postero-anterior roentgenogram six months following decortication.

JS3: Bronchogram showing "pseudo-bronchiectasis" in the left lower lobe due to compression.

JS4: Bronchogram six months following decortication showing good functional re-expansion with reversal of the previously apparent bronchial changes.

FIG. 12. Bronchogram revealing an undiagnosed occult fistula. This patient had had a left upper lobectomy followed by thoracoplasty (elsewhere) four years ago. He was discharged within one year and had been well until three months prior to admission when he developed cough, fever, malaise, and weight loss. Sputum was positive for tubercle bacilli on one culture and one smear.

AC1: Anteroposterior roentgenogram.

AC2: Admission bronchogram revealed "stump sinuses" at site of resected left upper lobe.

AC3: Following two weeks on antimicrobial and aerosol therapy, a repeat bronchogram revealed an occult bronchopleural fistula apparently communicating with the upper lobe bronchial stump and extending nearly to the diaphragm.

AC4: Following decortication with revision and closure of the upper lobe bronchus, a repeat bronchogram reveals the closure to be adequate and the absence of the empyema space.

recognized in compressed lung (84, 85). In these cases, one must view with caution the bronchographic finding of apparent bronchial disease in which there is no correlation to suggest clinical bronchiectasis. Such pulmonary tissue may be completely restorable functionally by decortication, as demonstrated in the present study. The decision regarding the surgical management of such lung tissue must be made at the operating table. If, following removal of the encompassing "peel," there is a re-expansion of the lung which on palpation is relatively soft and nonindurated, it is believed that decortication will prove successful in restoring function. Therefore, such lung tissue should not be resected merely on the basis of the preoperative bronchogram.

Pulmonary Tuberculosis with Previous Surgery

Nine of the 38 patients in this series with proved tuberculosis had had previous operations. This group consisted of patients with residual tuberculous disease, including reactivations and late complications. It also included those who underwent further bronchography after uncomplicated surgery. Fortunately, there have been few serious complications to evaluate.

All patients with tuberculosis in whom a surgical procedure is contemplated must be evaluated and treated on a strictly individual basis, particularly this group. Many have far advanced disease which may or may not be amenable to surgery. Others may have obscure complicating problems such as the masked or occult fistula. Moreover, in most of such patients, drug-resistant strains of tubercle bacilli and sometimes other drug-resistant bacteria have emerged to predominance (86, 87). The risk of extensive operations is high: less radical procedures directed to palliate or control selected pathologic conditions are often

FIG. 13. Progressive disease under thoracoplasty in a diabetic patient. Some infiltration has developed along the left heart border. The sputum is positive for tubercle bacilli.

The bronchogram reveals a large cavity in the lower lobe with ectasia of most of the basilar segments. This is not apparent on the standard roentgenogram.

Pneumonectomy was performed. The patient has noninfectious sputum and has been asymptomatic for one year.

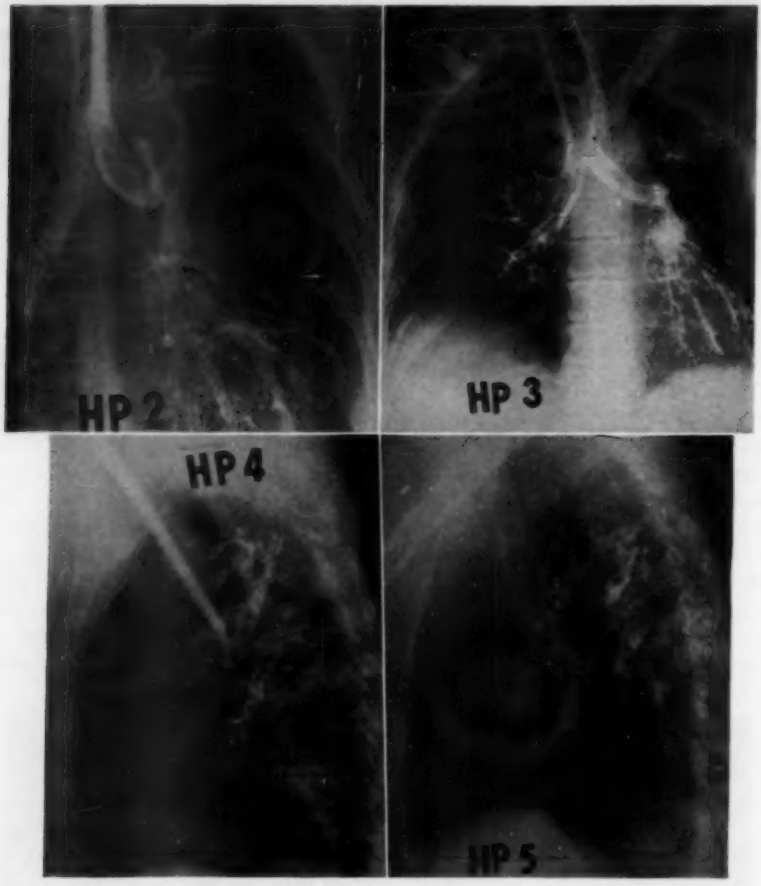

FIG. 14. Symptomatic bronchial torsion following segmentectomy. The patient underwent successful resection of the upper division (LUL) four years previously. For six months, she had had progressive, intractable cough. On forced expiration, there was a wheeze beneath the tip of the scapula followed by explosive cough. The bronchogram (HP2, HP4) revealed lingula lying in the cupola of the thorax with marked obstructive angulation of the left main and lower lobe bronchus; this explained the symptoms complex.

Following a three and one-half rib thoracoplasty, there was demonstrable improvement of the bronchial architecture (HP3, HP5) and complete relief of distressing symptoms.

indicated. Bronchography aids in identifying such lesions and in planning such treatment.

Bronchography has considerable screening value in the patient with progressive clinical disease following surgery. The demonstration of unsuspected empyema spaces with bronchial communication (occult fistula, figure 12) and

the differentiation of such lesions from cavities involving the superior segment of the lower lobe or other areas are often possible with Dionosil. Cavities in the lower lobes are more readily filled than in the upper, possibly due to gravity and/or the bronchiectasis which is frequently present (figure 13). With bronchography, it is also possible to demonstrate normal pulmonary patterns in areas which are obscured on the standard roentgenogram by postoperative changes.

Bronchial Torsion

The effect of thoracoplasty on bronchial distortion in advanced tuberculosis has been discussed previously. A type of postoperative major bronchial torsion is well illustrated in figure 14. The patient's complaint of severe, intractable, expiratory cough was relieved by a limited thoracoplasty.

Tuberculosis—Possible but Unproved

Saccular bronchiectasis, especially of the apical regions of the lung, is often the final result of chronic fibroid tuberculosis (11). More frequently than not this type of bronchiectasis may remain asymptomatic. It need not necessarily be removed simply because it is discovered *unless* repeated hemorrhages occur. If it is "tuberculous" bronchiectasis, i.e., active tuberculous infection in the ectatic wall, it may well represent the only source of infectious sputum or of recurrent hemoptysis, especially in elderly patients (85). Bronchoscopy is utilized

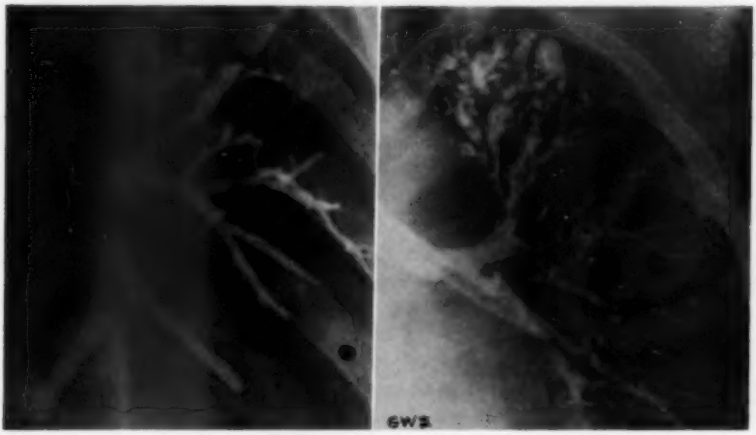

FIG. 15. The patient had a past history of tuberculosis with noninfectious sputum for eight years. Admission to the hospital was because of recurrent hemoptysis. Bronchoscopy showed narrowing of the apical posterior segment (LUL). The bronchogram demonstrates proximal segmental stenosis of the apical posterior segment of the left upper lobe.

On the spot film (GW1) the segmental bronchial stenosis and irregularity are well shown. The complete bronchogram shows the upper division bronchiectasis and a normal lingula. Bisegmentectomy (upper division, LUL) was performed. The patient has remained well.

in the evaluation of the source of hemoptysis, and it is often found that bronchography too is essential in the diagnosis of such lesions, particularly when tubercle bacilli are not demonstrable in the sputum (figure 15).

The present writers have seldom used bronchography in the evaluation of solitary nodules of the lung, as they are in agreement with Davis (88) that the diagnosis of such lesions should be made by early thoracotomy.

* * *

Many investigators have proved that valuable information may be given by bronchography in evaluating an individual patient's pulmonary tuberculosis. With the use of Dionosil Oily the inconvenience of retained radiopaque material and the potential dangers of iodized oil are avoided. The segmental bronchi and smaller bronchioles are readily demonstrated with this agent, which is a distinct advantage. Earlier and more frequent application of this technique should become customary. Contrast studies, possibly in conjunction with tomography, will help restore to roentgenography its justifiable position as the diagnostic method most closely approximating that of "the white light of surgery" (89).

The bronchographic observations in this study have been correlated with the pathology of pulmonary tuberculosis and some of the current bacteriologic and chemotherapeutic aspects of the disease.

Summary

From the evidence in this study, considered together with other workers' recorded experience, the following general conclusions seem justified.

Bronchiectasis in which terminal bronchostenosis of the segmental bronchus is demonstrated is usually the concurrent and early end-result of advanced parenchymal disease or of tuberculous endobronchitis. With such a bronchographic finding the question of persistent disease and of the viability of "nonculturable" tubercle bacilli becomes much more acute; the incidence of recurrence may be considered sufficiently high to warrant resection. This holds equally true for "initial" as well as for "re-treatment" patients.

Bronchiectasis in tuberculosis is evidence of previous or currently active disease in the segment involved. In the absence of terminal segmental stenosis, there is usually bronchiolar filling which indicates functioning lung with minimal disease. Whether or not these segments should be removed is questionable. The value of resecting or preserving such segments must be weighed according to the extent of other areas of involvement, the problems of postoperative space management, the susceptibility of the patient's tubercle bacilli to the available chemotherapeutic agents, and the patient's clinical course. At the present time the writers are not resecting additional segments on the bronchographic evidence of associated bronchiectasis alone.

Saccular bronchiectasis (either nonspecific or tuberculous), especially of the apical regions of the lung, is often the late result of "chronic

fibroid tuberculosis" and may represent a source of infectious sputum or of recurrent hemoptysis, especially in older patients. Such lesions may require surgical treatment.

The demonstration of cavities by direct filling with the bronchographic agent is sporadic and unreliable.

Bronchography is of proved value in the diagnosis and evaluation of major bronchial disease associated with adjacent lymphadenopathy.

Bronchography is of special value in evaluating the pathologic status of patients who have had previous surgery with recurrent or progressive disease. It has also proved useful in evaluating the effect of surgery in selected cases.

Bronchography is indicated prior to almost any surgical approach to the treatment of pulmonary tuberculosis. The preoperative delineation of anatomic and architectural anomalies, the demonstration of lobar, segmental, or sub-segmental involvement (especially in contracted areas of lung), and the occasional finding of an unsuspected, but frequently significant, condition (such as a spontaneous bronchopleural fistula or nontuberculous basilar bronchiectasis) may be apparent with no other diagnostic technique.

Bronchographic findings in pulmonary tuberculosis are informative and of value in planning resective surgery, especially in patients with drug-resistant infections, in the management of residual lesions, and in the evaluation of residual disease following prior operation.

Sumario

La Broncografía en la Tuberculosis Pulmonar: Estudios sobre Su Valor en los Enfermos Considerados para Cirugía

A base de los datos de este estudio, considerados junto con las observaciones de otros técnicos, las siguientes conclusiones parecen justificadas:

La bronquiectasia en la que se descubre broncostenosis terminal de un segmento bronquial suele ser el resultado final concomitante y temprano de una enfermedad parenquimatosa avanzada o de una endobronquitis tuberculosa. Con un hallazgo broncográfico de tal género, se vuelve mucho más aguda la cuestión de la existencia de enfermedad persistente y de la viabilidad de bacilos tuberculosos "incultivables"; puede considerarse la incidencia de recurrencias como suficientemente alta para justificar la resección. Esto reza por igual con los enfermos "iniciales" así como con los "re-tratados."

En la tuberculosis, la bronquiectasia es signo de enfermedad activa anterior o actualmente en el segmento afectado. A falta de estenosis segmental terminal, suele haber henchimiento bronquiolar que indica pulmón funcionante con enfermedad mínima. Es dudoso si deben o no resecarse esos segmentos. Hay que determinar el valor de conservarlos o resecarlos de acuerdo con la extensión de las otras zonas afectadas, los problemas de la atención postoperatoria del espacio, la susceptibilidad de los bacilos tuberculosos del enfermo a los productos quimioterapéuticos disponibles y la evolución clínica del sujeto. En la actualidad, los AA. no resecan otros segmentos exclusivamente a base de los signos radiográficos de bronquiectasia coexistente.

La bronquiectasia sacular (ya anespecífica o tuberculosa), sobre todo en las regiones del vértice del pulmón, es a menudo el resultado tardío de una "tuberculosis fibrosa crónica" y puede representar un foco de esputo infeccioso o de hemoptisis recurrente, en particular en los enfermos de mayor edad. Esas lesiones tal vez requieran tratamiento quirúrgico.

El descubrimiento de cavernas mediante el henchimiento directo con la substancia broncográfica es esporádico e inconstante.

La broncografía es de valor demostrado en el diagnóstico y la justipreciación de las broncopatías de mayor cuantía asociadas con linfadenopatía adyacente.

La broncografía es de valor especial para justipreciar el estado patológico de los pacientes operadoa antes que tienen enfermedad recurrente o evolutiva. También ha resultado útil para valuar el efecto de la cirugía en casos seleccionados.

La broncografía está indicada antes de casi todos los abordes quirúrgicos al tratamiento de la tuberculosis pulmonar. Con ninguna otra técnica de diagnóstico tal vez pueda llevarse a cabo la demarcación preoperatoria de las anomalías anatómicas y arquitectónicas, el descubrimiento de invasión lobular, segmental o subsegmental (en particular en las zonas contraídas del pulmón) y el hallazgo de vez en cuando de una situación insospechada, pero frecuentemente importante (tal como fístula broncopleural espontánea o bronquiectasia basilar no tuberculosa).

En la tuberculosis pulmonar, los hallazgos broncográficos son informativos y valiosos al planear resecciones quirúrgicas, sobre todo en enfermos que tienen infecciones farmacorresistentes, en la asistencia de las lesiones residuales y en la justipreciación de la enfermedad residual consecutivamente a una operación,

Resume

La bronchographie dans la tuberculose pulmonaire: Etude de sa valeur dans les cas considérés opérables

La confrontation des données de l'étude et l'expérience acquise par d'autres auteurs permet les conclusions suivantes:

La bronchectasie avec bronchosténose de la bronche segmentaire représente une séquelle précoce associée à l'altération avancée du parenchyme ou à une endobronchite tuberculeuse. En présence de ces signes bronchographiques, le problème présenté par la maladie prolongée et la viabilité des bacilles tuberculeux "non cultivables" devient beaucoup plus aigu; l'incidence de la rechute peut être considérée assez élevée pour justifier l'exérèse ceci est également vrai qu'il s'agisse de cas "initiaux" ou de "re-traitement."

Dans la tuberculose la bronchectasie est la preuve d'une activité antérieure ou actuelle de l'affection au niveau du segment intéressé. En l'absence de sténose du segment terminal, il y a habituellement une oblitération bronchiolaire qui montre que le poumon fonctionne et que les lésions sont minimes. L'exérèse ou la conservation de ces segments est matière à controverse. La valeur de la résection ou de la conservation de ces segments doit être pesée en tenant compte de l'étendue des lésions dans les autres segments affectés, des problèmes du traitement postopératoire des espaces residuels, de la sensibilité des bacilles tuberculeux du malade aux agents chimiothérapeutiques en usage et de l'évolution clinique du cas. Désormais, les auteurs s'abstiennent de réséquer des segments additionels lorsque les signes bronchographiques indiquent qu'il ne s'agit que de bronchectasie associée.

La bronchectasie sacculaire (non spécifique ou tuberculeuse), spécialement au niveau de l'apex pulmonaire, est souvent la conséquence tardive d'une sclérose pulmonaire chronique d'origine tuberculeuse et elle peut être une source d'expectoration infectieuse ou d'hémoptysies à répetition, surtout chez le vieillard. Le traitement chirugical de ces lésions est peut être nécessaire.

La démonstration de la presence d'excavations par l'injection directe d'une substance de contraste est inconstante et peu sûre. La valeur de la bronchographie est démontrée dans le diagnostic et l'évaluation de l'affection bronchite grave accompagnée d'adénopathie adjacente.

La bronchographie a une valeur particulière pour l'évaluation des conditions pathologiques chez le malade ayant subi une intervention chirugicale pour une affection récurrente ou progressive; elle s'est également révelée utile dans l'évaluation des effets de la chirurgie dans certains cas sélectionés.

La bronchographie est indiquée avant presque toute intervention chirugicale en relation avec la tuberculose pulmonaire. Cette démarcation préopératoire des anomalies anatomiques et structurales peut révéler des lésions lobaires, segmentaires ou sous-segmentaires (surtout dans les aires rétractées du poumon) et parfois une lésion insoupçonnée, mais souvent significative, qu'aucune autre méthode diagnostique ne saurait rendre apparente.

Dans la tuberculose pulmonaire, les résultats de la bronchographie sont instructifs et profitables dans la préparation du plan d'exérèse, surtout dans les cas ne répondant pas à la chimiothérapie, dans le traitement des lésions résiduelles et dans l'évaluation des lésions qui subsistent après une intervention chirugicale.

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THE SURGICAL MANAGEMENT OF GIANT CAVITARY TUBERCULOSIS[1, 2]

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Introduction

The problem of effective treatment for patients with giant cavitary tuberculosis has not been solved by the application of prolonged chemotherapy; nor have the majority of surgical measures proved to be completely satisfactory.

The giant "cavern" of older reports has long been one of the most alarming manifestations of advanced pulmonary tuberculosis. Defined as a tuberculous cavity 4 cm. or more in diameter, this lesion carries with it the threat of such morbid complications as pneumothorax with bronchopleural fistula, empyema, hemoptysis, tension cavity with compression of adjacent lung, and spread to the opposite lung. In addition, there is the strong probability that a giant cavity will result in a predictable medical failure if unaccompanied by some type of surgical treatment.

The purpose of the present study is to review the methods and results of surgical measures which have been advocated for giant cavities and to describe the writer's experience with these methods during five years of the chemotherapy era (1950–1955). Several cases will illustrate the impression that a combination of antituberculous drugs and pulmonary resection whenever possible is the most satisfactory treatment for giant cavitary tuberculosis.

Review of the Literature

Prior to the discovery of specific antituberculous drugs, surgical efforts were largely limited to the standard thoracoplasty. Botelho and co-workers (1), in studying the results of multiple-stage thoracoplasty for patients with cavities exceeding 4 cm. in diameter, found only 55 per cent of the patients with sputum negative for *M. tuberculosis* seven to twelve years after treatment.

Ranch (2) found an incidence of 5 per cent of patients with giant cavities among 640 patients with moderately and far advanced disease. In this group, 59 per cent of those who were untreated died within one year. Pneumothorax was successful in 20 per cent but was attended by many complications. On the other hand, thoracoplasty with or without Monaldi intracavitary drainage was satisfactory in approximately 75 per cent of the patients.

One of the earliest references (3, 4) to the surgical drainage of tuberculous lung cavities is found in a book by E. Barry (published in Dublin, in 1726). Although used sporadically in the next century, it remained for Monaldi in 1938 (5, 6) to effectively use the technique of intracavitary drainage for tuberculous cavities of the lung. Several workers

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(7) have been unable to duplicate Monaldi's fine results, although Woodruff and associates (8), in experience with 82 cases, report cavities that have remained closed in 40 cases for periods from eight months to four and one-half years. Hirsch (9) stated that only in exceptional cases was a complete cure observed with this method alone. The same writer, in reporting a total of 20,000 intracavitary injections of antituberculous drugs, believed that intracavitary drainage was of considerable help as a treatment preparatory to surgical collapse.

Rubin and Klopstock (10) believe that the type of disease is a major factor influencing the results of thoracoplasty in pulmonary tuberculosis. In 23 patients with giant cavities, reversal of infectiousness and cavity closure were accomplished in 69.5 per cent. In a follow-up study of this group only 60.8 per cent remained well after five years. Monaldi drainage was employed as a preliminary measure when a tension cavity was suspected. In the giant cavitary group the results were unpredictable, but in general these patients required more supplementary-stage revision thoracoplasties than patients with other types of disease.

One of the outstanding complications of thoracoplasty, particularly in the giant cavitary group, has been that of spread or exacerbation of disease. In reporting 317 patients treated with multiple-stage thoracoplasty in the pre-streptomycin era (1934 to 1944), Yang and Lees (11) found a 10.7 per cent incidence of disease spread or exacerbation. All seven of their cases of extensive spread of disease occurred in patients who had cavities with diameters greater than 4 cm. They concluded that the extent of the spread of disease increased directly with the diameter as well as the number of pulmonary cavities.

Ottosen and associates (12) contrasted the results of 42 thoracoplasties and 20 resections performed in the years 1945 to 1947. All patients had giant upper lobe cavities. Nearly half of both groups had active disease in the contralateral lung. Of the thoracoplasty group (41 cases followed three to four years), the disease in 58.9 per cent was arrested, with an over-all mortality of 36.7 per cent. In the resection group, the disease in 56.3 per cent was arrested. The over-all mortality was 25 per cent. Although neither group had the benefit of current prolonged chemotherapy, the writers believed the discouraging results were attributable more to the far advanced stage of disease than to the type of therapy.

Material

Thirty patients with either unilateral or bilateral cavities 4 cm. or more in diameter have been seen during the five-year period, 1950 to 1955. Among the entire group of pa-

TABLE 1

Surgical Treatment of 30 Patients with Giant Cavities

Procedure	Number of Patients
Monaldi drainage only	8
Thoracoplasty	
Standard 6–8 ribs	10
Subcostal plombage	5
Resection	
Primary	4
Secondary (preliminary Monaldi drainage)	3
Total	30

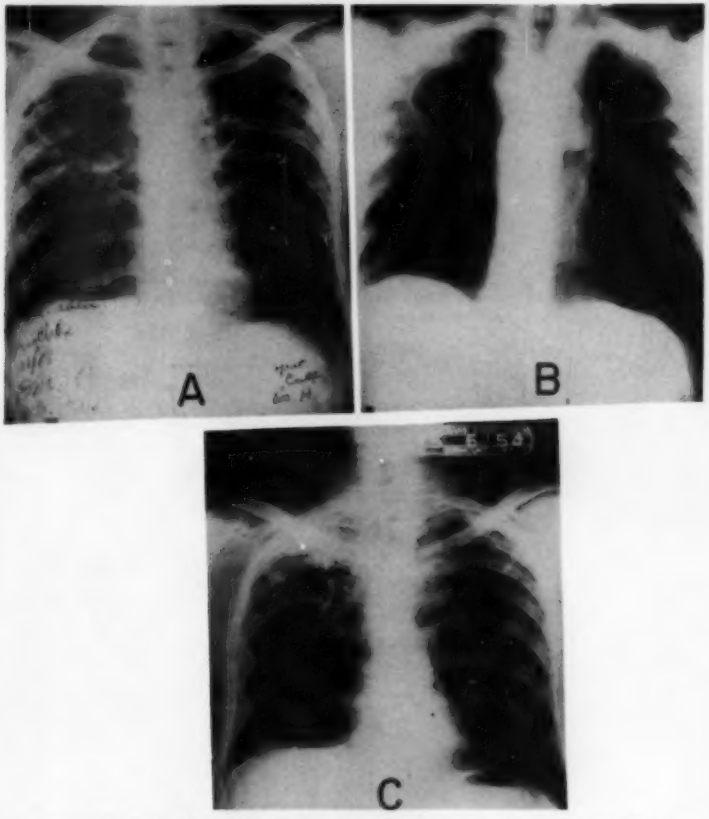
tients with cavitary tuberculosis studied during this interval, giant cavities represent an estimated incidence of 10 per cent.

A summary of the surgical treatment used in this group is given in table 1.

Observations

Four clinical cases that illustrate the variety and results of several types of surgical treatment are presented in figures 1 to 7.

In the 24 Monaldi intracavitary drainage procedures, 5 patients had minor complications. In 2 patients, a transient pneumothorax was induced, and in 2 patients it was not possible to enter the cavity despite repeated attempts. Ex-



Case 1 (H. P.)—*Monaldi Drainage and Bilateral Plombage Thoracoplasty*

Fig. 1*A*. A 31-year old white male with giant bilateral cavities: right, 7 by 6 cm.; left, 5 by 8 cm. The patient had an unsuccessful right pneumothorax in 1947. *B*. Planigram defining extent of cavities. *C*. Sputum remained positive for tubercle bacilli after several courses of streptomycin-PAS. Because of severe emphysema and extensive tuberculosis, a right Monaldi drainage was performed.

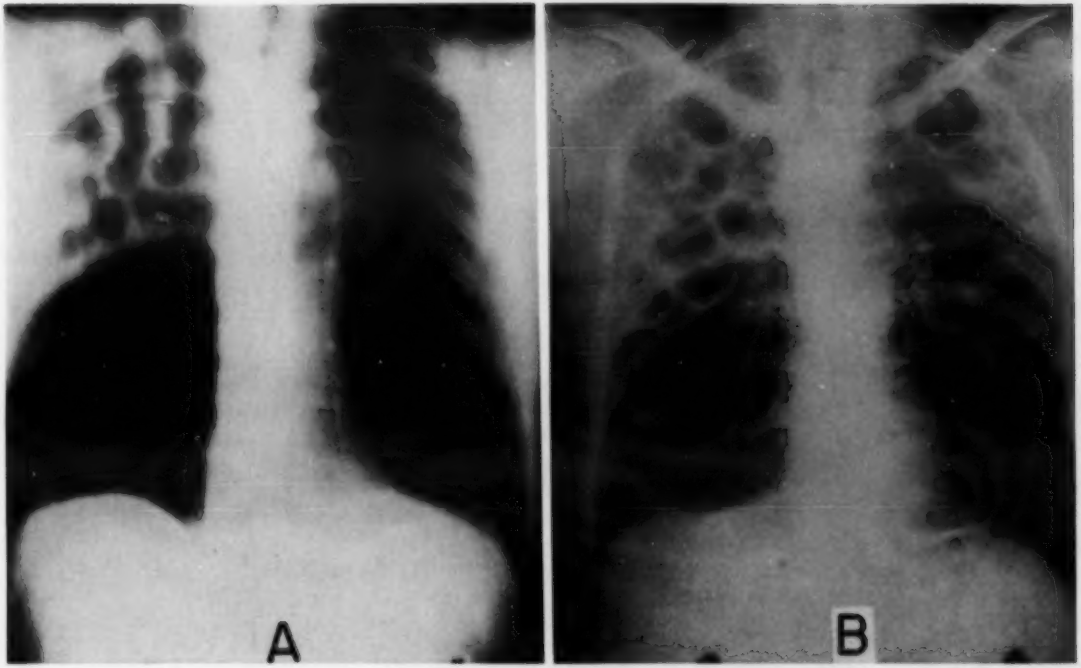
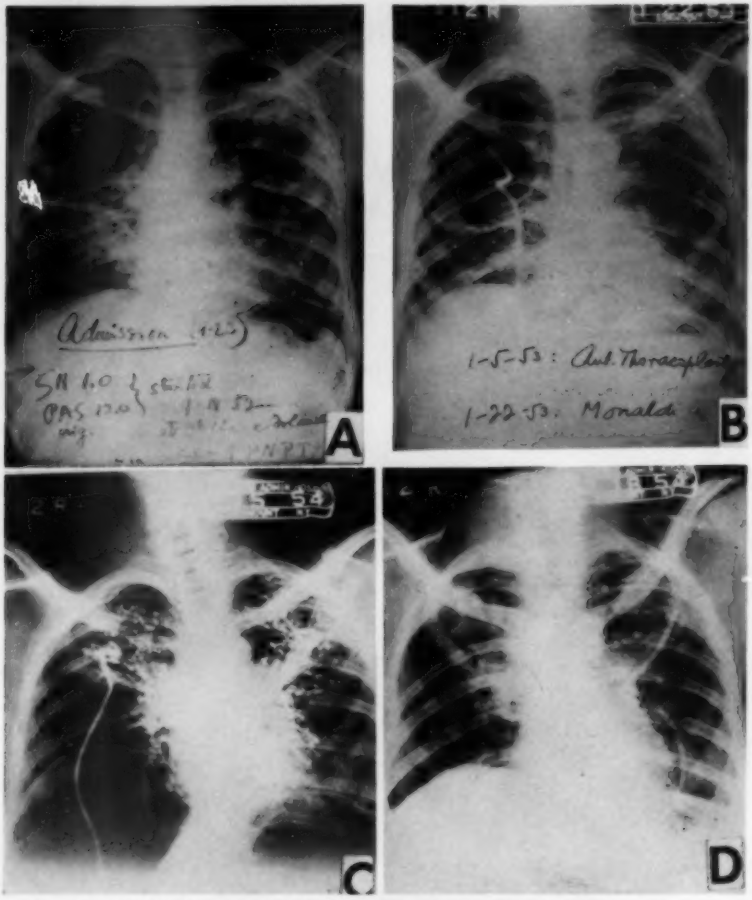


FIG. 2*A* (*Case 1*). Left Monaldi drainage was unsatisfactory. Resection was considered inadvisable, and a right plombage thoracoplasty was well tolerated. *B*. Following left plombage thoracoplasty, maximal breathing capacity was 40.5 liters per minute. Patient's sputum has been noninfectious for sixteen months.

cessive bleeding requiring suspension of efforts occurred in one case. In 3 patients there were minor difficulties with tuberculous anterior chest wall sinus tracts adjacent to the tube site. In 12 patients a definite decrease in cavity size was observed following several weeks of tube drainage. Five patients had no change, and in one patient the cavity became somewhat larger.



Case 2 (J. F.)—*Monaldi Drainage and Bilateral Segmental Resection*

FIG. 34. A 36-year-old Negro male was admitted to the hospital in a toxic, nearly moribund state with uncontrolled tuberculosis and diabetes, with bilateral giant tension cavities: right, 7 by 9½ cm.; left, 7 by 4 cm. Sputum was infectious (Gaffky VIII). *B*. The patient was treated with streptomycin-PAS. Following control of diabetes, a right Monaldi drainage was performed. A left Monaldi drainage was carried out in six months. *C*. Bilateral intracavitary bronchograms via Monaldi tubes. *D*. Following resection of two segments in right upper lobe. A similar resection was performed on left upper lobe.

As a consequence of thoracoplasty, one definite postoperative tuberculous spread of disease was noted. In the group of 5 patients who received extraperiosteal plombage, one late tuberculous infection of the subcostal space occurred, requiring removal of the plombage.

Other than two minor space problems, there were no major tuberculous complications in the group subjected to primary or secondary pulmonary resection.

The follow-up period in this group of 30 patients with giant cavities varies from six months to four years. As indicated in table 2, the long-term results are

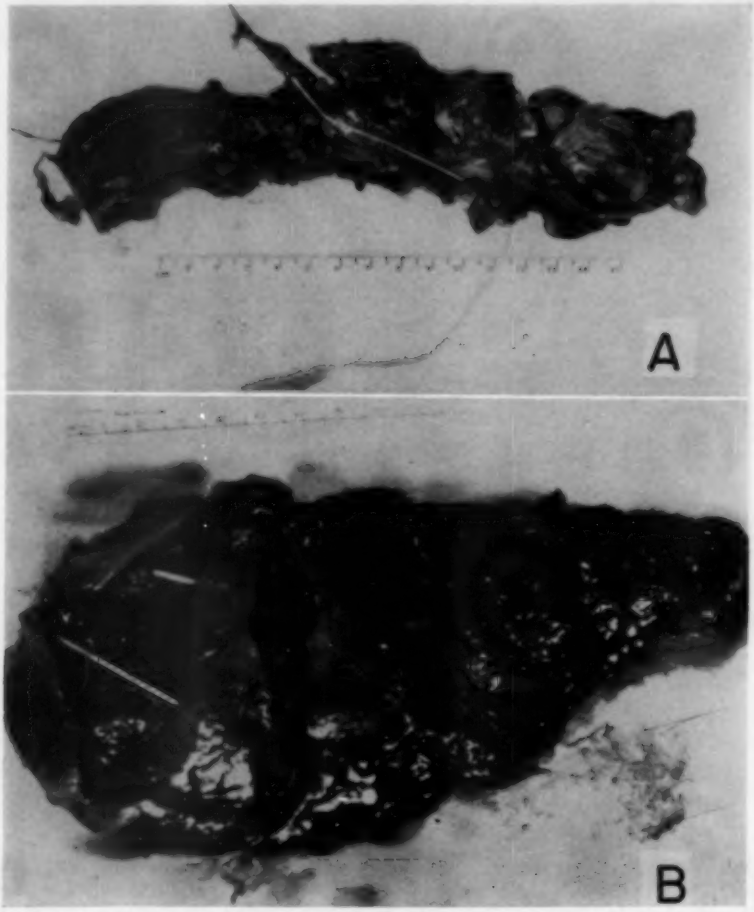
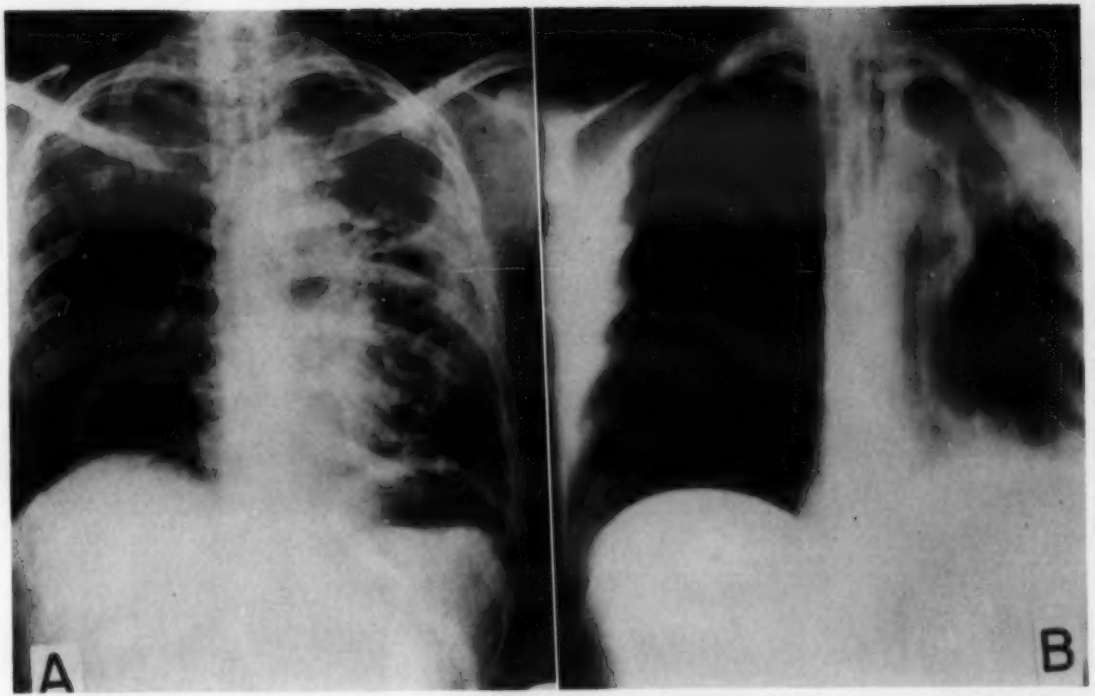


FIG. 4.*A* (*Case 2*). Resected specimen, right anterior and posterior segments of upper lobe. Probe in Monaldi tract. *B*. Specimen of apical posterior segment of left upper lobe. Large residual apical cavity. The patient is now well and working two years following surgery.



Case 3 (D. S.)—*Monaldi Drainage and Left Pneumonectomy*

Fig. 5*A*. A 38-year-old Negro male with a giant apical cavity, basal cavity in the left lung, and small apical cavity in the right upper lobe. Sputum heavily positive for *M. tuberculosis* on admission. *B*. Planigram showing extent of cavities. Previous chemotherapy with isoniazid-streptomycin was supplemented with viomycin.

not encouraging. Each of these patients, by definition, has moderately advanced or far advanced tuberculosis. Many were elderly males with complications in the form of vascular diseases. In 8 patients a Monaldi procedure alone was done as a palliative attempt, major surgery being contraindicated. Many of the patients were severe alcoholics; this factor further complicated the management of their disease. Two patients were transferred to mental hospitals while still receiving active treatment for tuberculosis.

Consideration of their present status shows that in 7 patients, about one-

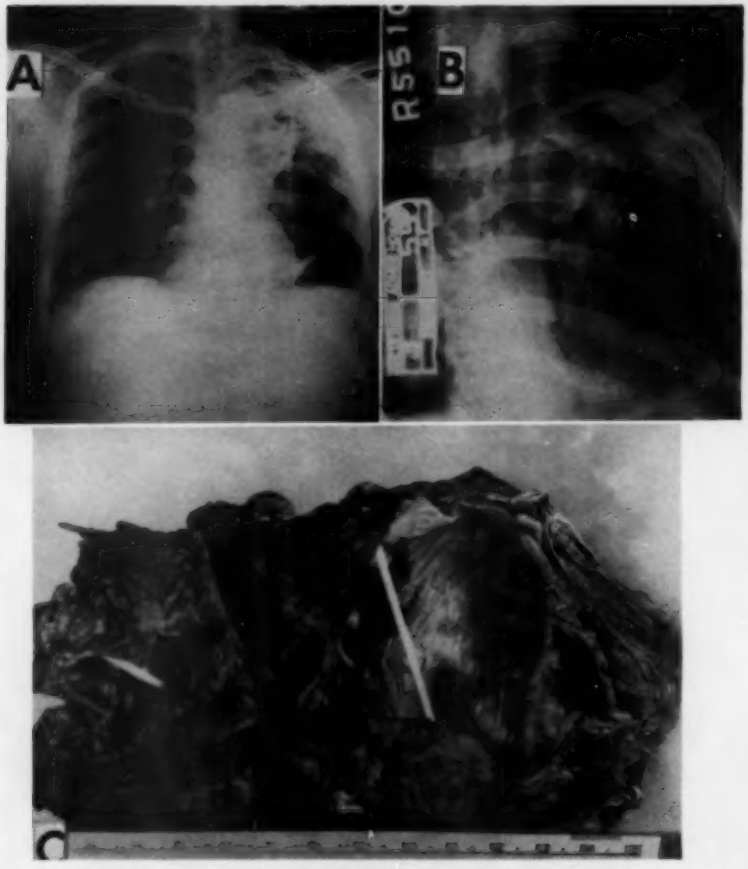
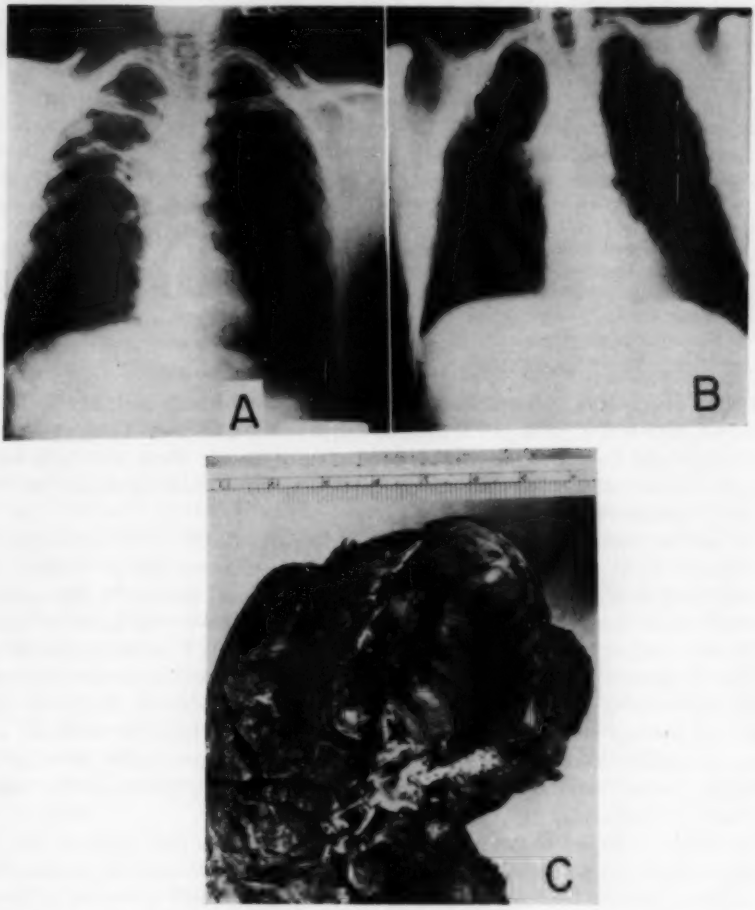


FIG. 6*A* (*Case 3*). Monaldi drainage of left upper lobe cavity. *B*. Decrease in cavity size following one month of suction drainage. Intracavitary bronchogram showing marked deformity of left bronchial tree. *C*. Sputum continued to be positive for *M. tuberculosis*, and a left pneumonectomy was performed. Specimen shows giant superior segment of basilar cavity and small apical cavity (Monaldi site). Specimen culture yielded bacilli resistant to all antituberculous drugs. Patient's disease relapsed six months following pneumonectomy from reactivation of cavity in right upper lobe.



Case 4 (R. L.)—*Primary Lobectomy*

FIG. 7*A* and *B*. A 29-year-old Negro male admitted to the hospital with sputum positive for *M. tuberculosis* and with a 5 by 7 cm. cavity in the right upper lobe. Primary chemotherapy with streptomycin-PAS was started. *C*. The patient had noninfectious sputum after four months on initial chemotherapy. A right upper lobectomy was carried out at six months. Specimen shows giant smooth-walled cavity which contained drug-susceptible bacilli. The patient's sputum has been negative for *M. tuberculosis* for nine months and he is now apparently well.

fourth of the original group, with giant cavitary tuberculosis, the disease has been converted to a satisfactory inactive state.

DISCUSSION

An evaluation of combined medical and surgical treatment for patients with giant cavitary tuberculosis must consider the advanced state of disease. The

TABLE 2

GIANT CAVITY TUBERCULOSIS FOLLOW-UP STUDY AND RESULTS

In hospital	
Disease under control	2
Disease stable (?)	3
Disease progressive	6
Discharged patients	
Transfer (active with infectious sputum)	2
Inactive	6
Unsanctioned discharge (active)	2
Probably inactive	2
Deaths	
Tuberculosis	6
Nontuberculous disease	1

majority of patients who manifest giant cavities, particularly with bilateral disease, are expected to be or prove to be so-called medical failures. Thus, prolonged chemotherapy may succeed only in producing a chronic state with continued infectiousness and the eventual emergence of organisms resistant to all available antituberculous drugs.

It has not been demonstrated that the Monaldi procedure has any intrinsic palliative benefit. On the contrary, the psychologic effects of being "wedded" to a tube and drainage system for many months is incalculable. In the present writer's small experience with Monaldi drainage there have been no cures unless drainage was followed by thoracoplasty or resection. This is not a condemnation of the Monaldi operation, as it is recognized that the details of the technique used were not as strictly followed as those originally described. However, with the exception of the occasional patient with a tension type of cavity or one raising copious secretions, presumably from the cavity, there seems to be no indication for intracavitary drainage even as a preparatory maneuver for thoracoplasty or resection.

It has been stated that preliminary Monaldi drainage may improve the results of thoracoplasty by aiding the extent of collapse in the paravertebral gutter or minimizing any tendency for contralateral spread. There is no real evidence to support this contention. It is the present writer's belief that the results of thoracoplasty in such an instance are due largely to the amount of disease and the physical nature of the underlying lung, technical details of thoracoplasty, and effectiveness of drug therapy, rather than to the aforementioned factors.

Pulmonary resection with the opportunity for palpation of the lung and selective removal of the cavity-bearing area appears to be the ideal procedure. If the policy is adopted of considering all patients with giant cavitary tuberculosis as predictable medical failures when first seen, resection may be accomplished within the first four to six months of original chemotherapy and maximal benefit obtained.

There should be little concern over leaving residual noncavitary disease in the lower lobe or contralateral lung. The success of antituberculous drugs in con-

trolling closed (noncavitary) lesions has been well demonstrated. Providing the exudative component has receded under appropriate drug combinations, removal of the cavity-bearing area should be the major consideration. Effective sterilization of the cavity or clearing of diseases immediately adjacent to the cavity are unlikely. The surgical handicap of operating on prolonged disease in a salvage situation, after drug control is uncertain or nonexistent, is considerable and, in many instances, could be avoided by a more aggressive medical and surgical approach in the early stages of tuberculosis.

Summary

One of the most ominous manifestations of advanced tuberculosis, the giant cavity (4 cm. or more), has resulted in the death of more than 50 per cent of patients when untreated by surgical measures.

A series of 30 patients with giant cavitary tuberculosis is presented. They were treated with various combinations of chemotherapy and surgical measures. No permanent arrests of disease were obtained in 8 patients treated with Monaldi drainage alone. In the remaining 22 patients thoracoplasty or resection with or without preliminary Monaldi drainage was satisfactory in 7 (one-third) of the group. It is believed that the results are indicative of the advanced stage of the disease and of the type of patients with the disease rather than a reflection of the surgical method.

The most consistent treatment results have been obtained by thoracoplasty, with disease arrest rates approaching 75 per cent in the pre-chemotherapy period. Thoracoplasty failures or late relapses after five- to ten-year intervals, however, now average 10 per cent (13). The advantages and safety of pulmonary resection when effective antituberculous drug coverage is available should make this the treatment of choice. Proceeding on the assumption that giant cavitary tuberculosis will be a therapeutic failure on nonsurgical treatment, early pulmonary resection should yield results similar to those for all varieties of cavitary tuberculosis.

Sumario

La Asistencia Quirúrgica de la Tuberculosis de Cavernas Gigantes

Una de las manifestaciones más ominosas de la tuberculosis avanzada, la caverna gigante (de 4 cm. o más), ha culminado en la muerte de más de 50 por ciento de los enfermos que no fueron tratados con providencias quirúrgicas.

Se presenta una serie de 30 enfermos que tenían tuberculosis de cavernas gigantes y fueron tratados con varias combinaciones de quimioterapia y medidas quirúrgicas. No se obtuvieron estacionamientos permanentes en 8 enfermos tratados exclusivamente con el drenaje de Monaldi. En los 22 enfermos restantes la toracoplastia o la resección con o sin previo drenaje de Monaldi resultaron satisfactorias en 7 (la tercera parte) del grupo. Parece que estos resultados son indicativos de la etapa avanzada de la enfermedad y de la clase de sujetos que padecen de ella más bien que reflejo del método quirúrgico.

Los resultados terapéuticos más constantes fueron los obtenidos con la toracoplastia, con coeficientes de estacionamiento que se acercaron a 75 por ciento en el período prequimioterapéutico. Sin embargo, hoy día promedian 10 por ciento (13) los fracasos de la toracoplastia o las recidivas tardías al cabo de plazos de cinco a diez años.

Las ventajas y la inocuidad de la resección pulmonar cuando se cuenta con protección eficaz con drogas antituberculosas deben convertir este tratamiento en el de elección. Partiendo de la suposición de que la tuberculosis de cavernas gigantes es un fracaso médico predecible, la resección pulmonar temprana debe rendir resultados semejantes a los conseguidos en todas las variedades de la tuberculosis cavitaria.

Resume

Traitement chirurgical de la tuberculose à cavernes géantes

Une des manifestations les plus dangereuses de la tuberculose avancée, la caverne géante (4 cm. ou plus) a causé la mort de 50% des malades quand l'intervention chirurgicale a été omise.

Une série de 30 malades porteurs d'excavations géantes est présentée. Le traitement a consisté en diverses combinaisons chimiothérapeutiques et en interventions chirurgicales. Chez 8 malades, le drainage de Monaldi n'a pas procuré une stabilisation permanente. Chez les 22 autres malades, la thoracoplastie ou la résection, avec ou sans drainage, a donné des résultats satisfaisants dans 7 cas (⅓ du groupe). L'auteur est d'avis que ces résultats démontrent qu'ils se rapportent au stade avancé de la tuberculose et à un certain type de tuberculeux, plutôt qu'il ne permettent l'évaluation de la méthode chirurgicale.

Les résultats thérapeutiques les plus constants ont été obtenus avec la thoracoplastie, les taux de stabilisation atteignant près de 75% avant la période de chimiothérapie. Néanmoins, les échec de la thoracoplastie ou les rechutes tardives, au bout de cinq à dix ans, atteignent maintenant en moyenne 10% (13).

Les avantages et la sécurité de l'exérèse pulmonaire, quand on dispose de la protection d'agents tuberculostatiques efficaces, doivent la faire considérer comme le traitement de choix. En admettant que les lésions cavitaires géantes constituent un risque d'échec, la résection pulmonaire précoce permet d'envisager des résultats analogues à ceux qui sont mentionnés dans toutes les variétés de tuberculose cavitaire.

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MILIARY TUBERCULOSIS[1]

A Review of Sixty-Eight Adult Patients Admitted to a Municipal General Hospital

J. PARK BIEHL

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Introduction

Of the various forms of tuberculosis, miliary tuberculosis has shown the most dramatic reversal in prognosis through the use of specific antimicrobial therapy. It is doubtful that any patients with the acute disease survived before the introduction of streptomycin in 1945. By 1956, however, the combined experience of the U. S. Veterans Administration hospitals was that more than 90 per cent of those treated survived (1).

In the course of observing patients admitted to the medical wards of the Cincinnati General Hospital, certain features of miliary tuberculosis have become apparent. In general, these features are inadequately described in the literature. Consequently, it has become clear that a new perspective is advisable in considering the patient with miliary tuberculosis and that the characteristics of the disease should be re-examined, particularly as they pertain to diagnosis.

The present report consists of an analysis of the clinical and laboratory findings and the results of therapy in a series of 68 patients with miliary tuberculosis consecutively admitted to the hospital.

The term *miliary tuberculosis* denotes massive invasion of the bloodstream by tubercle bacilli so as to produce small tubercles in large numbers in certain organs and miliary lesions in the lung. A distinction is made between the acute and more chronic forms of this type of tuberculosis. In the latter form, the process continues over a longer period of time, allowing more extensive necrosis, confluence, and fibrosis to occur. The term "miliary" is preferred in this context to "hematogenous" or "disseminated," since the latter terms are frequently used to designate localized blood-borne tuberculous disease, such as renal tuberculosis or lymphatic tuberculosis, whereas "miliary" has the desired connotation of widespread dissemination. In the present report the term "miliary disease" indicates any disease roentgenographically simulating miliary tuberculosis.

Case Material

The material upon which this report is based consists of 68 patients consecutively admitted to the hospital during the years 1951 to 1956 in whom the final diagnosis was miliary tuberculosis. Most of the patients have been treated and/or subsequently followed, when they survived, by the writer. Included also are 7 patients in whom the diagnosis was made only at autopsy over the same period of years. In most instances the diagnosis was confirmed bacteriologically or pathologically; in 7, the diagnosis was not proved. The 7 improved patients are included on the basis of the clinical picture. In some cases this included the occurrence of typical tuberculous meningitis, the presence of tuberculous granulomata in liver biopsy specimens, and the response to specific therapy.

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Twenty of the 68 patients (30 per cent) had tuberculous meningitis in addition to miliary tuberculosis. As this complication tended to obscure the implications of the miliary tuberculosis, it will be kept discriminate in certain of the analyses.

Follow-up data have been obtained in all surviving patients, either by direct examination by the writer or by correspondence with the patient or his physician. The status of all but 2 patients is known as of February, 1956. These 2 are known to have been well four and four and one half years after the illness was first treated.

Observations

Clinical Features

Predisposing factors: Race has been considered to be a factor in the reaction of the host to tubercle bacilli. It has been stated that hematogenous dissemination tends to occur more readily in non-whites than in whites and that various hematogenous forms of tuberculosis may be more common in the U. S. Negro than in the U. S. white. This greater frequency in non-whites has occurred in the experience of the present writer with respect to serosal tuberculosis, tuberculous adenitis, and chronic disseminated tuberculosis, as well as in miliary tuberculosis. In the present series, only 10 patients were white; the remaining 58 were Negroes. In contrast to this, the adult population of Cincinnati in 1955 consisted of only 16.5 per cent Negroes. The less favorable sociologic and economic conditions of life of the Negro in this country clearly play a great part in his predisposition to tuberculosis in general; yet the same conditions are also imposed upon segments of the white population. It is likely that the recent living conditions of white persons and of Negroes entering the Cincinnati General Hospital do not differ greatly. About half of the hospital admissions represent Negroes (51.5 per cent during the years 1951–1955), indicating approximate numerical equality in the two groups from which patients are drawn. Thus, it is likely that the high incidence of adult miliary tuberculosis in the Negro represents a real racial characteristic having to do with the control of dissemination of tuberculosis. The valid objections to this conclusion include the question of the living conditions of the patients in either group in the remote past, especially of those at the time of the primary infection when initial dissemination probably occurred.

The *age* distribution of the patients is shown in figure 1. It is particularly significant that 28 (41 per cent) of the patients were sixty years of age or older. The incidence according to age groups as it relates to the distribution of the total population of Cincinnati in these age groups is also shown in figure 1. The elevation of the line graph as age sixty is reached indicates a twofold increase in the incidence of miliary tuberculosis over what would be expected. The higher incidence of miliary tuberculosis in the aged has not generally been recognized. It was low in Auerbach's large series of cases examined at autopsy (2).

The distribution of patients by *sex* reveals twice as many males (47 patients) as females (21 patients). Of interest is a tendency for the females to be younger than the males: of 22 patients less than forty years old, 14 were women. Five of the 14 were either pregnant on admission or had been pregnant within 10 months of their admission to the hospital. It appears, therefore, that pregnancy may be a

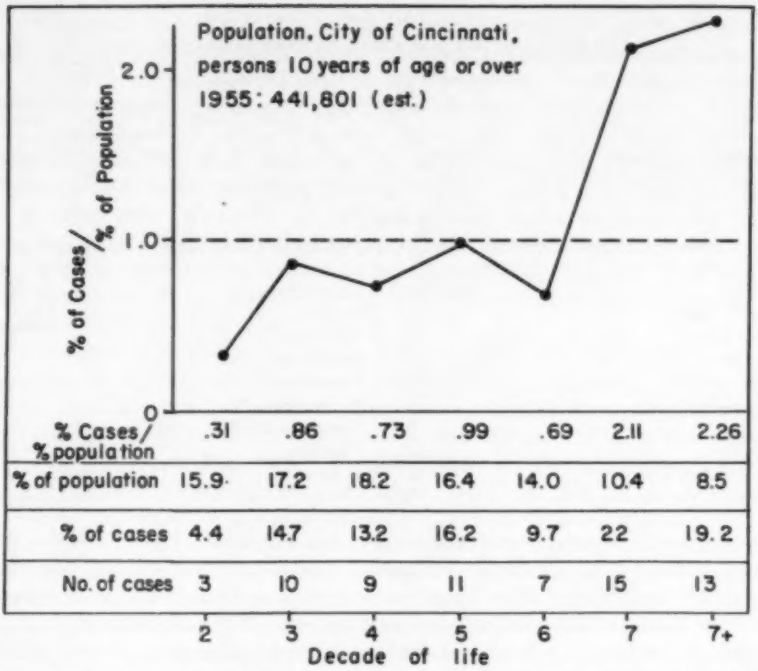
Population, City of Cincinnati,
persons 10 years of age or over
1955: 441,801 (est.)
% of Cases / % of Population
2.0
1.0
0
% Cases/ % population .31 .86 .73 .99 .69 2.11 2.26
% of population 15.9 17.2 18.2 16.4 14.0 10.4 8.5
% of cases 4.4 14.7 13.2 16.2 9.7 22 19.2
No. of cases 3 10 9 11 7 15 13
2 3 4 5 6 7 7+
Decade of life

FIG. 1. The effect of age on the incidence of miliary tuberculosis. The percentage of the cases falling into various age groups is compared with the normal percentage of the population falling into the same age groups. In the line graph, if age had no influence, the line would be horizontal at 1.0. The graph indicates in this situation a twofold increase in the incidence of miliary tuberculosis in persons more than sixty years of age.

factor in producing dissemination. This relationship of pregnancy to miliary tuberculosis has been observed by others (3).

No other predisposing factors could be detected in the backgrounds of these patients. Only 5 were described as alcoholic. There was no significant trend with regard to trauma, recent illness other than tuberculosis, or physical debility. Diabetes occurred only once in the group.

Symptoms: The onset of the symptoms of miliary tuberculosis was characteristically insidious, and the duration of the illness prior to admission was frequently obscure. The stated length of illness is given in table 1, in which the range of 3 to 15 weeks includes most of the cases. A few were fulminating, while in others the disease had apparently been producing symptoms for more than 3 months. The symptoms most commonly elicited are tabulated in table 2. Most prominent among these are cough, weight loss, weakness, anorexia, and night sweats. As a group, these symptoms could obviously be caused by any of a large group of diseases. Additional symptoms which were more commonly related to

TABLE 1

DURATION OF ILLNESS PRIOR TO HOSPITALIZATION

	Weeks							
	1	2	3	4-5	6-10	11-15	16+	Unknown
Number of patients	2	3	15	8	14	12	5	9

TABLE 2

SYMPTOMS ENCOUNTERED IN 62 PATIENTS ABLE TO RELATE A HISTORY

Cough	39	Headache	11
Weight loss	38	Malaise	6
Weakness	34	Delirium	6
Anorexia	26	Abdominal pain	6
Night sweats	23	Chest pain	4
Feverishness	22	Hemoptysis	4
Chills	15	Upper respiratory infection	3
Dyspnea	14	Diarrhea	2
Nausea and vomiting	12	Aching	2

the presence of meningitis included headache and delirium. The course of symptoms in those with meningitis suggested that miliary tuberculosis preceded meningeal involvement by a considerable period of time. For, the symptoms common to those with and without meningitis were present over most of the period of the illness; when headache and delirium supervened, it was usually just before admission to the hospital.

Of the 6 patients who complained of abdominal pain, 3 had peritonitis or retroperitoneal adenitis in addition to the miliary tuberculosis.

Physical findings: There were few physical signs present in all of the patients other than fever and moist pulmonary sounds, either rhonchi or coarse rales. The latter were present in 57. Of interest was the absence of palpable splenomegaly in all but one patient (*Case 63*). Splenic enlargement was present in 3 of the 24 patients on whom necropsy was performed. In *Case 3* the spleen weighed 325 gm. In *Case 44* it weighed 970 gm., and was the site of multiple infarcts. In *Case 63* it weighed 1,214 gm., due to the presence of chronic caseous tuberculosis. Palpable hepatic enlargement was only occasionally noted. Splenic and hepatic enlargement is usually observed in children with miliary tuberculosis. This is in contrast to the situation in adults, in whom the otherwise uninvolved spleen does not become enlarged in the presence of miliary tubercles alone.

History of previous tuberculosis: In only 3 cases was there a previous history of known tuberculous disease. In *Case 40*, a 26-year-old woman had previously had tuberculous peritonitis at the age of eight years. Cervical and axillary adenitis, pulmonary tuberculosis, and tuberculous polyserositis had previously been present in *Case 13*. This same patient again entered the hospital, as *Case 52*, with miliary tuberculosis three years later.

Concurrent tuberculosis: Active tuberculosis, other than miliary and meningeal,

was recognized clinically in 22, or approximately one-third, of the patients. Generally only one such focus was detected per patient. A notable exception was a patient (*Case 13*), who entered with peripheral lymphatic, peritoneal, abdominal lymphatic, and epididymal tuberculosis in addition to the miliary form of the disease.

The additional forms of tuberculosis found clinically in the 22 patients are shown in table 3. In the remaining 46 cases there was no clinical evidence of other foci of tuberculosis. When the 12 patients with meningitis are eliminated, the remaining 34 represent one half of the total series in which miliary tuberculosis was not accompanied by any other form of tuberculosis detected by ordinary clinical methods. Most of these (27 patients) lived long enough to permit a reasonably thorough diagnostic study.

The results of the autopsies provide more complete data on the presence of accompanying tuberculosis, but must be considered as applying only to a selected portion of all patients with miliary tuberculosis, namely, those not surviving. Concurrent active tuberculosis was found in 18 of 24 patients upon whom necropsy was performed. The sites of infection found are shown in table 3. In 3 patients examined at autopsy no tuberculosis other than miliary was detected. Three other patients had had only miliary and meningeal tuberculosis.

These data serve to illustrate the facts that only a minority of all cases of miliary tuberculosis arise in patients with pulmonary tuberculosis and that no other evidence of active tuberculosis may commonly be found in many patients with miliary tuberculosis. The obvious implication is that miliary tuberculosis is to be found in persons not hitherto considered tuberculous and not necessarily in the patients referred to the thoracic specialist.

TABLE 3

THE INCIDENCE OF ACTIVE TUBERCULOSIS IN FORMS OTHER THAN MILIARY OR MENINGEAL

Site	Clinical Demonstration	Necropsy Finding	Total*
Number of patients	22	18	35
Lung	7	9	13
Lymph nodes			
deep	1	8	8
superficial	3	0	3
Pleura	6	2	8
Peritoneum	3	3	6
Genitourinary	3	3	6
Adrenals	0	4	4
Pericardium	0	3	3
Bone	2	1	3
Spleen	0	2	2
Liver	0	1	1
Brain	0	1	1
Endometrium	1	0	1
Larynx	1	0	1

* Some sites were shown both clinically and at necropsy.

The number of cases of miliary tuberculosis occurring as an isolated clinical entity also indicates the frequent inconspicuousness of the chronic disseminated or pulmonary tuberculosis from which the miliary dissemination originates and the inadequacy of our diagnostic methods for detecting these foci. It is clear also that most cases of miliary tuberculosis represent but one facet of the total tuberculous disease present. In the treatment of such patients, therefore, it is necessary to realize that older chronic active caseous disease, as well as the acute dissemination, is probably present in the patient.

The occurrence of adrenal tuberculosis in 4 of 24 patients examined at autopsy is in keeping with the relatively high incidence of adrenal involvement in patients with disseminated forms of tuberculosis. Such a relationship is important to bear in mind, as adrenal destruction may remain latent for long periods of time until a suitable stress provokes a fatal adrenal crisis. Appropriate steps should be taken in patients with disseminated forms of tuberculosis to exclude the presence of adrenal insufficiency. On the other hand, adrenal tuberculosis is uncommon in pulmonary tuberculosis without demonstrable dissemination.

Hypersensitivity to tuberculin: The skin reaction to tuberculin (PPD) was tested in 26 of the patients close to the time of admission to the hospital. Hypersensitivity was demonstrated in only 16 (61 per cent). Of these, 10 reacted to 0.00002 mg. of PPD, and 6 reacted only to the second-strength, or 0.005 mg. Eight of the 10 nonreactors died. One survivor later was found to react to 0.005 mg. of PPD after 12 months of therapy.

Leukocyte count: Emphasis is placed on the fact that the leukocyte count is normal in most cases of miliary tuberculosis. Patients in this series with miliary tuberculosis and no meningeal involvement had normal leukocyte counts in 43 of 46 cases. The 3 exceptions showed counts as high as 14,200 per cu. mm. The average for the series was 6,600, and in 6 the count was less than 4,000 cells per cu. mm. Eight of 19 patients with complicating meningitis had counts in excess of 11,000. All but one of these died within a few days of the leukocyte determination.

Chest roentgenogram: The demonstration of miliary lesions in the chest roentgenogram remains essential for the clinical diagnosis of miliary tuberculosis. The films of 6 patients in this series, however, did not reveal such lesions. Five of the patients survived only a few days, and the diagnosis of miliary tuberculosis was proved at autopsy in all 6. In 2 of these patients (*Cases 18* and *36*), death occurred within a few days after the normal roentgenogram was obtained, and necropsy revealed typical miliary tubercles in the lungs. In 2 others (*Cases 8* and *65*), far advanced pulmonary tuberculosis obscured the miliary process. In another patient (*Case 63*), a month elapsed between the normal chest roentgenograms and necropsy demonstration of tubercles, and in the sixth (*Case 21*), the film was not suitable for excluding the presence of miliary lesions.

In each of the remaining 63 cases, nodular lesions were demonstrated on the roentgenogram. These were distributed diffusely throughout both lung fields and were indistinguishable roentgenographically from the miliary lesions produced by numerous other causes.

Diagnosis

The differential diagnosis of miliary disease has been made more difficult by the demonstration that many different agents are capable of causing the appearance of miliary lesions in the chest roentgenogram. Felson (4) has enumerated forty causes of acute miliary disease of the lung, recognizing that only sixteen are not exceedingly rare conditions. Frequently, moreover, more chronic causes must also be considered, since an unrelated acute febrile illness may be superimposed on chronic miliary disease, such as silicosis. In spite of these considerations, however, at the Cincinnati General Hospital most patients presenting with miliary disease have been proved to have miliary tuberculosis. Other diseases considered most frequently in the differential diagnosis have been sarcoidosis and silicosis. Miliary histoplasmosis in the adult has not been encountered by the present writer except for a local epidemic previously described by Feldman and Sabin involving a number of men who had cleaned out a pigeon loft (5).

The desirability of bacteriologic or histologic proof of the tuberculous etiology of cases of miliary disease is obvious. In this age of specific chemotherapy one can no longer wait for the diagnosis to become apparent as the disease progresses untreated; nor is it advisable to initiate specific antituberculous therapy unless steps have first been taken to produce bacteriologic or histologic confirmation. For, once started, there rarely is any alternative but to extend therapy for at least a year's time. The physical and economic distress thus experienced by the patient may be of great importance to him, as well as the social stigma and the possible threat that he has latent tuberculosis which will remain with him for the rest of his life.

Criteria for diagnosis: An attempt has been made to obtain the diagnosis in the members of this series either by the detection of *M. tuberculosis* or by the demonstration of a grossly caseous granulomatous lesion. In 9 of the 68 patients, special tests were not performed to prove the diagnosis of miliary tuberculosis. In one case (*Case 48*) the tests were omitted because the patient's symptoms were considered to be so typical of miliary and meningeal tuberculosis that it was not considered necessary. The other 8 were not tested, either because miliary tuberculosis was not suspected at the bedside or because the patient lived for only a few hours after arriving at the hospital. In the remaining 59 patients the diagnosis by the above criteria was obtained in 49 (83 per cent).

Sources of the diagnosis: In order to isolate *M. tuberculosis*, cultures and microscopic examinations were made of samples of sputum, gastric washings, urine, cerebrospinal fluid, bone marrow aspirates, and serosal exudates. The techniques employed are summarized as follows:

Sputum was digested with hypochlorite, concentrated, and examined microscopically for acid-fast rods. *Gastric aspirates* were digested in 2 per cent sodium hydroxide and centrifuged for a total of one hour, neutralized, and cultured on Löwenstein-Jensen slants. *Urine*, collected for 24 hours, was decanted to concentrate the sediment, which was digested and seeded similarly to gastric aspirates, and was also examined microscopically. *Cerebrospinal fluid* was centrifuged and decanted. The sediment was examined microscopically and seeded in its entirety on slants of culture medium. *Bone marrow aspirates* were sub-

jected to digestion in various forms and seeded on slants. *Serosal exudates* were digested, centrifuged, examined microscopically, and cultured similarly to urine. *Biopsy material* was subjected to routine histopathologic procedures and was stained also for acid-fast rods.

Complete "batteries" of these procedures were not performed on each patient, particularly when one or two procedures proved diagnostic before the others were performed. The results in the 59 patients examined are shown in table 4 and are summarized in table 5. It is noteworthy that the sputum and/or gastric contents of 30 (61 per cent) of 49 patients examined was positive for *M. tuberculosis*.

Seven of 28 patients had urine cultures positive for *M. tuberculosis*. Autopsy in 2 of these patients 18 and 60 days after the start of therapy revealed no renal tuberculosis other than miliary lesions. In both, however, many of the miliary lesions were older than in more acute cases, and showed more caseation than usual. Pyelograms of 3 others revealed no roentgenographic evidence of renal tuberculosis. The dissemination in these 7 patients was thought to be more chronic. It seems likely that their urine yielded tubercle bacilli because of the increased opportunity for the renal tubercles to become caseous and slough into the calyces. Whether such patients should be considered as having had renal tuberculosis in the usual sense is questionable. It is possible that bacilli could have been passed with the glomerular filtrate without having produced local disease, as claimed by Wallgren (6). Any renal disease that was present in these 7 patients was limited to the same diffuse miliary process that occurred in other organs, and responded in a similar manner if the patient survived.

The detection of tubercle bacilli by bone marrow aspiration was disappointingly infrequent in this series; only 4 of 24 examinations were positive. It is to be expected, however, that the procedure would be more sensitive if the specimen were not digested prior to seeding onto the culture media.

The cerebrospinal fluid was positive for tubercle bacilli in most cases in which meningitis was present and once in 26 cases in which meningitis was not present. Serosal exudates and biopsies of focal disease, such as lymph nodes, peritoneum, or endometrium, when obtained for study, were usually positive on culture for tubercle bacilli.

Liver biopsy: Punch biopsy is a most sensitive and useful procedure for the demonstration of granulomatous disease involving the liver. Besides tuberculosis, however, such lesions are also commonly seen in such other systemic diseases as sarcoidosis, brucellosis, and early syphilis, and have been described in Hodgkin's disease, infectious mononucleosis, histoplasmosis, malaria, beryllliosis, and leprosy (7–11). It is impossible to identify hepatic granulomas as tuberculous on a morphologic basis alone unless *M. tuberculosis* is demonstrated. Thus, liver biopsy cannot be expected to be diagnostic per se, except insofar as it provides material for bacteriologic study.

The frequency and significance of such lesions in tuberculosis has recently been the subject of study. Ringleb (12) found 123 instances of "retothelial nodules" in liver biopsies performed on 464 patients with tuberculosis, an

TABLE 4

SOURCES OF DIAGNOSTIC DATA

Case Number	Sputum	Gastric Washings	Urine	Cerebrospinal Fluid	Bone Marrow	Other*	Autopsy
1		−	−	+			
2†							+
3†							+
4		−		−		LNB +	
5	−						
6		+			+		
7			+	−		PF +	
8	+						+
9	+			−			
10		+	−	−			
11			+	+	−		+
12		+	−	+	−		+
13	+	+	+	−	+		
14	−	−	−	−	−		
15		−		+	−		
16	+		−	−	−		
17		−			−		
18†							+
19		+	+	+			+
20							
21							+
22†				−	−	AF −	
						PB +	
23	−		−	+	−		
24		+	−	−			
25				+			+
26	+	+			−		
27				+			
28	+		+	−	−		
29		−		+	−		+
30		+	−	−	−		
31		+	−	−	−		+
32			−		−		+
33	+	+		−	−		
34							+
35		+	−	−		PF +	
36							+
37		−		−			+
38	+			+			
39				−		AF +	
40	−	−		+	−		
41				+			+
42	−	−	−	+			
43		+	−	−			
44			−	−			+
45		−	−	−	−	LNB +	
46	−					EB +	
47	+						

TABLE 4—CONTINUED

Case Number	Sputum	Gastric Washings	Urine	Cerebrospinal Fluid	Bone Marrow	Other*	Autopsy
48							
49	−	−	−	−	−		
50	−	+		−	−		
51	−	+	−	−	−		
52	−	+	+				
53	+		−	−	−		
54		+	−	−	+		+
55	−			−	+		+
56	+		+				
57	−	+	−	−			
58		−	−				
59	−	−	−	−			+
60	+			−			
61				+			+
62	+			−			
63							+
64		+		−		LNB +	
65		−				PF +	+
66		+				PF +	
67		−	−	−			
68		+		+			+

* LNB = Lymph node biopsy
PF = Pleural fluid
AF = Ascitic fluid
PB = Peritoneal biopsy
EB = Endometrial biopsy.
† Miliary tuberculosis not suspected before death.

incidence of 26.5 per cent. Olderhausen and associates (13) found an identical incidence of granulomata in 384 cases of nonhematogenous pulmonary tuberculosis. Haex and Van Beek (14), in 189 cases of various pulmonary and disseminated tuberculosis, found caseous or noncaseous tubercles in 176 (93 per cent). How many of the lesions in these reports were caseous was never precisely given. Buckingham and associates (15), in 128 cases of tuberculosis, found caseating granulomata in 23 per cent and histiocytic nodules in 26 per cent. The infectious nature of the smaller noncaseous lesions is not established and has been doubted. Whatever their origin, it is clear that the incidence of hepatic granulomata in tuberculosis far exceeds the incidence in miliary tuberculosis, and granulomata are thus not diagnostic of miliary tuberculosis. The demonstration of caseous granulomata, however, may still be useful in certain situations. The occurrence of the caseating granuloma in a patient with miliary disease makes it reasonable to assume that the roentgenographic findings are also due to the granulomata, thus reducing the number of possibilities.

Liver biopsy was performed in 7 of the patients in this series; in each case, examination of the tissue revealed caseous miliary granulomata. In none were acid-fast bacilli demonstrated microscopically.

TABLE 5

INCIDENCE OF DIAGNOSTIC INFORMATION FROM VARIOUS SOURCES

Source	Number of Patients Examined	Number Positive	Per Cent Positive
Sputum	26	13	50
Gastric washing	35	20	57
Sputum and/or gastric washing	49	30	61
Urine	29	7	24
Bone marrow	25	4	16
Cerebrospinal fluid			
Patients with meningitis	18	14	78
Patients without meningitis	27	1	—
Serosal exudate	6	5	—
Biopsy			
Lymph node	3	3	—
Peritoneum	1	1	—
Endometrium	1	1	—

Instances in which the diagnosis was not correctly made before death: Six patients died with diagnoses other than miliary tuberculosis. In all 6, the incorrect diagnoses are attributed to the failure of the roentgenogram to reveal the characteristic lesions (in one, the reading of miliary disease on the roentgenogram was made post mortem). Three of these patients lived only one to two days after admission; 3 survived 10, 32, and 60 days. The true diagnosis was revealed only at autopsy. Most of these patients were thought to have had advanced carcinomatosis while alive.

Therapy

Antituberculous chemotherapy is clearly indicated when miliary tuberculosis is suspected. Procedures for the isolation of *M. tuberculosis* should be started promptly, but need not be the cause of delay of more than three days in starting antimicrobial therapy. These procedures are designed, then, not so much to give a basis for the initiation of therapy, but rather justification, in some cases, of the continuation of therapy over a long period of time. In the severely ill patient in whom treatment is started before all bacteriologic specimens are obtained, material for culture is still worth obtaining, even after the start of such therapy. This is particularly true in the case of sputum and gastric washings.

It should be realized that in treating miliary tuberculosis two phases of the infection are to be considered. Miliary dissemination represents hematogenous seeding from an older caseous focus of infection, which is often occult. Dissemination is therefore an episode in the course of chronic tuberculosis and represents only a part of the total disease process involved in the therapeutic problem. One therefore must design treatment for both the acute and chronic infection. As in the case of other forms of tuberculosis, the treatment period

should exceed considerably the time required for disappearance of the acute process.

Bed rest: The period of confinement to bed in this series was extremely variable. Ten patients were allowed to be up and about the ward within 2 to 12 weeks after the start of treatment. These were selected because of the acuteness or mildness of the infection or because of a rapid response to therapy. Other patients were confined for longer periods, especially when the response to therapy was slow.

Antimicrobial therapy (table 6): Streptomycin with *p*-aminosalicylic acid (PAS) was given to 10 patients in doses of 1–2 gm. and 12 gm., respectively. Isoniazid with streptomycin was given to 45. The dose of isoniazid was 300 mg. daily to 19 patients. Eight received a dose of 10 mg. per kg., and 18, a dose of 20 mg. per kg. daily. In the latter two regimens, pyridoxine, 5 mg. per kg. per day, was added in order to prevent peripheral neuritis, which is otherwise a common side effect of isoniazid at this dose (16). Usually after 2 to 4 months, the streptomycin dosage was reduced to 1 gm. twice weekly; and the isoniazid, to 300–400 mg. daily. Thirteen patients received no chemotherapy.

Chemotherapy was never deliberately discontinued before 6 months. Twenty-four patients were treated for a total of 1 to 2½ years.

Drug resistance of the strains of tubercle bacilli to streptomycin, PAS, or isoniazid *in vitro* was not detected in this series, and was not suspected in any case other than *Case 19*. This patient, who also had meningitis, appeared to be refractory to therapy with streptomycin and PAS. The patient's strain of tubercle bacilli was not tested for drug susceptibility. Although the emergence to predominance of streptomycin-resistant tubercle bacilli in miliary tuberculosis has been clearly established, a similar phenomenon with isoniazid has not been observed. This indicates that at least in certain cases the patient may respond satisfactorily to therapy with isoniazid alone. In actuality, this was demonstrated by Clark and associates (18) in 10 patients with isolated miliary tuberculosis.

TABLE 6

ANTIMICROBIAL THERAPY EMPLOYED: 55 PATIENTS WITH MILIARY TUBERCULOSIS

1. Drug regimens	
Streptomycin–PAS	10
Isoniazid–streptomycin	45
Isoniazid, 300 mg. daily	19
Isoniazid, 10 mg. per kg. daily	8
Isoniazid, 20 mg. per kg. daily	18
2. Duration	
Less than 3 weeks	19
3 weeks to 3 months	4
3 months to 6 months	5
6 months to 12 months	3
12 months or more	24

Results of Therapy

General: The symptoms of miliary tuberculosis frequently were rapid in their disappearance after the start of antimicrobial therapy. The surviving patients almost always were symptom free, within the restrictions of bed rest, before a few weeks of therapy had elapsed. Notable exceptions were several patients with complicating meningitis, in whom an "intrathecal tuberculin" type of reaction occurred, similar to those described by Meyer and associates (17).

Fever: Defervescence began within a week in almost all cases and was complete after an average of 5 weeks. One patient showed a rapid fall in temperature to normal in 2 days. Others required as much as 10 weeks before the fever gradually reached normal. Return of the temperature to normal was gradual in all cases. Whether the patient received isoniazid or streptomycin made no perceptible difference in the speed of defervescence in the series of Clark and associates (18). The results in the present series were in agreement on this point.

Roentgenographic change: A series of roentgenograms sufficient to show improvement in the miliary lesions was available for study in 27 of the cases. In each film the severity of the miliary lesions was graded as 0, 1, 2, or 3-plus. The criteria for grading were arbitrary yet are reasonably clear in the minds of the reviewers, and the evaluations have been reproducible on subsequent reevaluation. A zero, or normal, film was so judged in context with the earlier films showing the lesions.

The trends of change with antimicrobial chemotherapy are shown in figure 2. Each patient in the series is represented by a single line. It is obvious that the roentgenographic response to treatment was varied. Significant improvement, indicated by an improvement of one degree (such as from 3+ to 2+), was delayed for more than 2 months in some; but in 9 patients significant improvement occurred within 3 weeks. It may be seen from the figure that the greatest amount of improvement occurred during the first 6 weeks, judged by the frequency of steeper slopes in that period. It is believed that in those patients showing worsening after the start of therapy the density formed by the

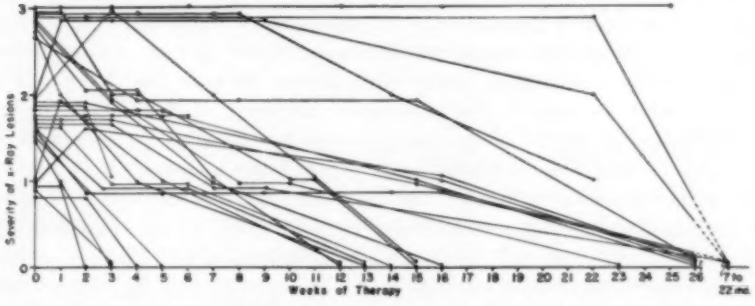

FIG. 2. Roentgenographic improvement during chemotherapy—27 cases.

TABLE 7
SURVIVAL RATES

	Miliary Tuberculosis Only	Miliary and Meningeal Tuberculosis	Total
Number of patients receiving any chemotherapy	37	18	55
Number of survivors, any chemotherapy	23	7	30
Per cent surviving, any chemotherapy	62	39	54.5
Number of patients receiving therapy of more than 7 days' duration	32	14	46
Number surviving, 7 days' therapy	23	7	30
Per cent surviving, 7 days' therapy	72	50	65

lesions was to a large extent altered by the restoration of normal hydration in the patient.

A return to normal roentgenographic findings occurred in all patients but one who were followed for a sufficiently long period. Seven to 22 months was required in 4 patients, while in 5 others the chest films were normal within 5 weeks. The majority (13 of 23 patients) were normal by the end of 16 weeks. From the figure it may be seen that the period required to show complete clearing correlates roughly with the initial degree of involvement. Speed of clearing also correlates with the promptness of the first appearance of improvement. Of 13 patients showing significant roentgenographic improvement within the first 3 weeks of therapy, 11 had showed complete clearing by the end of 16 weeks. Of 11 whose films remained unchanged or showed worsening during the first 3 weeks, 9 required more than 16 weeks for the chest films to show complete clearing.

Slowness of roentgenographic clearing was seen in the older patients; 7 of the 9 patients 56 years of age or older required more than 6 months to show roentgenographic clearing. There was no correlation between the duration of the illness prior to treatment and the rapidity of clearing.

Survival: Data concerning the survival of the entire group of 68 patients are shown in table 7. Fifty-four per cent of those receiving as little as one dose of streptomycin or isoniazid survived. If one eliminates those who died within 7 days of the start of chemotherapy, it may be seen that 65 per cent survived. A less favorable outlook is apparent when tuberculous meningitis is also present. Of those without meningitis who received therapy for more than 7 days, 77 per cent survived. In figure 3 the cumulative mortality of the 55 who received chemotherapy is shown. Of significance is the fact that few patients died who were able to survive the first month of therapy. Those who died after one month died under special circumstances. One patient (*Case 19*) died at 60 days, having had tuberculous meningitis with cerebrospinal fluid cultures persistently positive for tubercle bacilli despite therapy with streptomycin and PAS. At autopsy the miliary disease was regressing, and it is believed that he died primarily of tuberculous meningitis. In *Case 68*, a 76-year-old woman had previously been

severely incapacitated by cerebral arterial thrombosis. She entered the hospital with miliary tuberculosis, which responded to therapy, and died of terminal bronchopneumonia. Miliary lesions could not be demonstrated at autopsy, 60 days after the start of therapy. In *Case 59* in which the patient died 78 days after the start of therapy, death resulted from recurrent pulmonary embolism and infarction. The patient of *Case 16* died 172 days after the start of therapy after having shown complete roentgenographic clearing of the miliary tuberculosis. This patient, previously described in detail (19), apparently developed encephalopathy due to isoniazid or streptomycin. A 70-year-old patient (*Case 54*) died 230 days after the start of therapy. He never had regained consciousness, and it was shown at autopsy that he had a large tuberculoma of the left parietal lobe. The immediate cause of death was streptococcal septicemia.

In each of these 5 patients, death after one month of therapy was due primarily to causes other than miliary tuberculosis.

The introduction of isoniazid into the therapy of tuberculosis has produced qualitative differences in the response of the patient in regard to disseminated disease. Clark and associates (18) and others subsequently have drawn attention to the fact that the therapy of miliary tuberculosis with isoniazid prevents the supervention of tuberculous meningitis during therapy. This was estimated to be expected in as many as 50 to 66 per cent of all adult patients treated in the era prior to isoniazid (20). The present study bears out this apparently universal protective effect in that, in 29 patients without meningitis at the start of isoniazid therapy, meningitis did not emerge during therapy or thereafter.

There is little further basis for determining other effects of the addition of isoniazid to the therapy of miliary tuberculosis, since 45 patients were treated with regimens including isoniazid, and only 10 were treated with other drugs. Four of the latter died, while 22 of the 45 who received streptomycin with isoniazid died. When the patients with meningitis are excluded, the figures are materially changed; 11 of 29 patients with miliary tuberculosis without meningitis died, despite therapy with streptomycin and isoniazid. While 4 of these

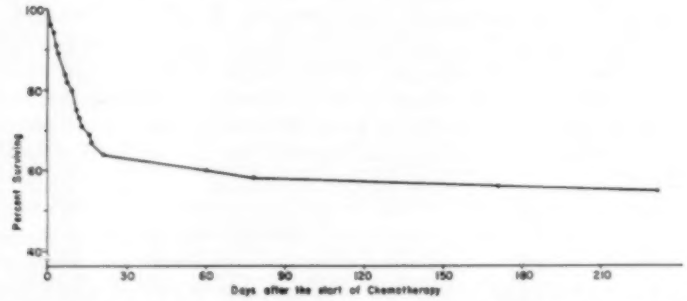

FIG. 3. Cumulative mortality, 55 cases of miliary tuberculosis, 1951 to 1956. The mortality falls sharply in those who survived the first month of therapy.

11 died under rather special circumstances (*Cases 68, 59, 16,* and *54* above), the death of the other 7, nonetheless, indicates that the therapy of this disease in adults has not yet reached a satisfactory stage of development.

Features associated with an unfavorable prognosis, in addition to the presence of meningitis, include the presence of active pulmonary tuberculosis and the absence of cutaneous hypersensitivity to tuberculin. Smith, Stevens, and Piles (21) noted the unfavorable influence of active pulmonary tuberculosis in tuberculous meningitis. Thirteen patients in the present series had active pulmonary tuberculosis and only one survived (*Case 60*). This exception is perhaps the more remarkable in having also shown signs of tuberculous meningitis and tuberculous epididymitis. The patients with pulmonary disease were first discovered through having developed miliary tuberculosis and had not previously received antituberculous chemotherapy.

Nonreactivity to tuberculin (PPD) was found in 10 patients, 9 of whom were bacteriologically proved to have tuberculosis. Eight of these 10 died.

Relapses: Two of these patients have relapsed. One (*Case 13*) again developed acute miliary tuberculosis several years after the apparent cure of the first episode (becoming, thereby, *Case 52*). The other (*Case 7*) refused regular therapy, and after 19 months of intermittent chemotherapy developed tuberculous meningitis and died.

Summary

From a study of 68 adult patients with miliary tuberculosis, the following may be concluded.

Miliary tuberculosis is a disease most likely encountered in the general hospital or in the practice of any physician. The illness frequently lends itself to therapy in hospitals other than tuberculosis sanatoriums, and later in the home. It is thus of increasing interest to the internist and the general practitioner.

An increased proportion of the cases occur in the aged and in Negroes. It is commonly not associated with detectable tuberculosis elsewhere in the patient.

The diagnosis seldom is suggested by routine examination; it is strongly suggested by the roentgenographic demonstration of miliary nodulation. The main differential possibilities in the adult are tuberculosis, sarcoidosis, and silicosis. Further diagnostic aids are culture of pulmonary secretions and urine and biopsy of suggestive disseminated lesions. Proof may be achieved bacteriologically or by the demonstration of gross caseation. Liver biopsy may be of value in confirming the granulomatous nature of this disease. It is seldom diagnostic per se.

Therapy should be directed toward the miliary process and to the underlying caseous process from which it originated. This process commonly is not apparent clinically. Therapy is successful according to the extent of disease. Most of those not surviving will die within the first month. Relapse in this series did not occur during chemotherapy. Relapse one to six years after the start of therapy has occurred in only one adequately treated patient.

A relatively poor prognosis is indicated in those patients who also have fibro-

caseous pulmonary tuberculosis and is suggested in those failing to show tuberculin hypersensitivity.

Sumario

Granulia. Estudio Clínico de Sesenta y Ocho Casos en Adultos Admitidos en un Hospital General Municipal

De este estudio de 68 casos de granulia en personas adultas, cabe deducir los siguientes puntos:

La granulia es una enfermedad que se observará más probablemente en el hospital general o la clientela particular. La dolencia se presta frecuentemente a tratamiento en hospitales que no son sanatorios para tuberculosos y después en el hogar, por lo cual reviste interés cada vez mayor para el internista y el médico general.

La proporción mayor de los casos de granulia corresponde a las personas de edad y a la raza de color negro. No se asocia comúnmente con tuberculosis descubrible en otra parte del cuerpo. Rara vez sugiere el diagnóstico el examen corriente; sí lo sugiere elocuentemente el descubrimiento radiográfico de nódulos miliares. Las principales posibilidades diferenciales en el adulto son tuberculosis, sarcoidosis y silicosis.

Otros auxiliares en el diagnóstico son el cultivo de las secreciones pulmonares y la orina y la biopsia de lesiones diseminadas indicativas. Puede obtenerse la comprobación bacteriológicamente o por el hallazgo de caseación macroscópica. La biopsia hepática puede resultar de valor para confirmar la naturaleza granulomatosa de la dolencia, pero raramente es diagnóstica por sí sola.

La terapéutica debe encaminarse al proceso granúlico y al proceso caseoso subyacente del cual parte el otro. Este proceso por lo regular no es evidente clínicamente. El éxito de la terapéutica depende de la extensión de la enfermedad. La mayoría de los enfermos que no sobreviven fallece durante el primer mes. En esta serie no hubo recidivas durante la quimioterapia. En un solo enfermo tratado adecuadamente se observó una recidiva de uno a seis años después de iniciar la terapéutica.

Queda indicado un pronóstico relativamente malo en los enfermos que tienen además tuberculosis pulmonar fibrocaseosa y sugiere lo mismo la falta de signos de hipersensibilidad a la tuberculina.

Resume

Tuberculose miliaire. Etude clinique chez l'adulte de soixante-huit cas admis dans un hôpital municipal général

Cette étude de soixante-huit cas de tuberculose miliaire chez des adultes permet les conclusions suivantes:

La tuberculose miliaire est une maladie qui a des chances d'être rencontrée dans un hôpital général ou en clientèle privée. La maladie peut souvent être traitée dans d'autres hôpitaux que les sanatoria pour tuberculose et ensuite à domicile. Elle suscite donc de plus en plus l'attention de l'interniste et du praticien.

Une proportion croissante de cas de tuberculose miliaire se produit chez le vieillard et le nègre. La forme miliaire n'est pas habituellement associée à une tuberculose décelable en un autre point de l'organisme du malade. Le diagnostic est rarement suggéré par l'examen ordinaire; il est fortement indiqué en présence de nodules miliaires sur les radiogrammes. Les principaux diagnostics différentiels chez l'adulte sont la tuberculose, la sarcoïdose et la silicose.

D'autres moyens diagnostiques sont représentés par la culture des sécrétions pulmonaires et de l'urine et la biopsie des lésions disséminées suspectes. La preuve peut être obtenue bactériologiquement ou par la démonstration d'une caséification évidente. La biopsie du foie peut être utile en confirmant la nature granuleuse des lésions; elle ne permet que rarement le diagnostic.

Le traitement doit s'adresser au processus miliaire et au processus de caséification qui

l'a créé. Ce processus n'est habituellement pas apparent cliniquement. Le succès du traitement dépend de l'étendue des lésions. La plupart des malades qui ne survivent pas meurent au cours du premier mois. Dans cette série il n'y a pas eu de rechutes durant la chimiothérapie. La rechute, qui se produit entre un mois et six ans après l'instauration du traitement, n'a été observée que chez un seul des malades traité de façon adéquate.

Chez les malades qui sont également atteints de tuberculose pulmonaire fibro-caséeuse le pronostic est relativement mauvais et il doit être réservé chez les malades anergiques à la tuberculine.

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AN ANALYSIS OF 3,485 TUBERCULOSIS CONTACTS IN THE CITY OF EDINBURGH DURING 1954–1955[1]

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INTRODUCTION

The introduction of effective antituberculous chemotherapy has brought about a dramatic change in tuberculosis. Death rates have been greatly reduced and in many areas morbidity rates have also declined, although much more slowly. It is now regularly expected that even patients with far advanced disease will be rendered noninfectious by the use of long-term chemotherapy. As a result of this, the known pool of infection in the community has been greatly reduced. For example, in Edinburgh at the beginning of 1954 there were between 300 and 400 known infectious cases of tuberculosis outside of the hospital, and a year later this number had been reduced to 45. With the reduction in the pool of infectors, the chance of infection for the individual is similarly lessened. In Edinburgh in 1954, 30.2 per cent of "non-contact" children aged thirteen to fourteen years were tuberculin positive; in 1955 this percentage was 24.3 per cent; and by 1956 it had fallen to 20.1 per cent (1). The result of this is that contact with a known source of infection assumes greater significance, and contact examination is more important than ever before.

In the pre-chemotherapy era, a contact of a case of tuberculosis was in many instances exposed to continuous or intermittent infection for many years. At the present time this should rarely happen.

For these reasons it is believed that it is important to assess the present state of the examination of persons previously exposed to tuberculosis and to investigate some of the problems encountered in running a clinic for such tuberculosis contacts.

The importance of contact examination in tuberculosis has been accepted for many years. It is probable that the first organized scheme for the examination of contacts was in Edinburgh following the opening of the original Tuberculosis Dispensary by Sir Robert Philip in 1887. Sir Robert instituted the "march past" of the family contacts of known cases, and attempts were made to diagnose the disease at an early stage and to institute treatment. It is apparent from Philip's later writing that he continued to teach and emphasize the need for contact supervision, but it is also apparent that the general recognition of the importance of this work was later lost. In the editorial columns of *Lancet* of June, 8, 1929, it is stated "one of the least profitable duties of the Tuberculosis Officer is that which concerns the examination of contacts" (2).

The importance of contact examination and of the increased tuberculosis morbidity

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among contacts was stressed by various authors (3–6) over the succeeding years. Thompson (7), Seideman (8), and Chadwick and Pope (9) quoted evidence suggesting that the initial contact examination is the most important one.

Various writers have also estimated the infection rates among contacts, as opposed to the morbidity. It is generally accepted that contacts of tuberculous patients show a higher proportion of infected persons at each age than the noncontact group. The difference between contacts and noncontacts should become more pronounced as the general level of infection in the community falls.

Bluhm (10) found that gastric lavage positive cases did not seem to cause an excess of infection in their contacts. Shaw and Wynn-Williams (11) suggested that patients with sputum positive for tubercle bacilli only on culture (negative on microscopy) are for practical purposes noninfectious, and these writers stated "the 'culture positive' patient may be treated in the same way as the 'sputum negative' one from the Public Health aspect." Blahd and associates (12) and Van Zwannenberg (13) both showed that the danger of infection was greatest with persons with large numbers of tubercle bacilli in the sputum, but that even persons with relatively scanty bacilli in the sputum produced infection in their contacts in a higher proportion than persons with arrested lesions and no tubercle bacilli in the sputum. Hertzberg (14), in his recent detailed and exhaustive study, has made careful measurements of the infection rates among contacts of infection-sources grouped on bacteriologic, roentgenographic and clinical bases. He has shown a wide gradation of infectiousness in the different types of disease.

Little seems to be known about the acceptability of contact examination. It is apparent that in the past many contacts were never seen at all, or were seen only when serious symptoms arose. With the increased radiologic facilities available in most countries it is now feasible to obtain roentgenograms of all household contacts and to cast the net beyond the home to include social, leisure, and occupational contacts. It is important to assess the practical difficulties in this work in order that the resources of the tuberculosis service may be directed in the most profitable way. Chadwick and Pope (9) and Cuthbert and Drimmie (15) achieved attendance of 74 per cent and 78.6 per cent, respectively, of contacts, but both found the attendance was lower in the older age groups. Tyrrell and Smith (16) in a special investigation in Glasgow succeeded in examining 413 of 415 home contacts, and thus showed that almost complete acceptance of contact examination could be achieved. The secret of their success seemed to have been the personal approach by the physician himself, including visits to the household. Hertzberg (14) was also able to obtain a high percentage of contact attendance.

The present survey was undertaken to try to provide up-to-date information on the significance of contact with cases of tuberculosis under present-day conditions of effective treatment with the consequent disappearance of tubercle bacilli from the sputum. In addition, an attempt was made to provide some information on the problem of organization for the examination of contacts.

The city of Edinburgh has a population of about 470,000 persons, and the tuberculosis service is administered from one center, the Royal Victoria Dispensary. Consequently, it is possible to collect information about cases of tuberculosis and tuberculosis contacts for the whole city.

METHODS

A *contact* was defined as a person who, in the opinion of the health visitor or the physician, had been in significant contact with a known case of tuberculosis. Contacts were divided into *household* and *nonhousehold* groups.

The *index case* of a contact was the case of tuberculosis which first set in train the events leading to the attempt to examine that contact. The *source case* of a contact was the case of tuberculosis judged by the physician to have been the most probable source of infection for that particular contact, after considering all of the information available about the various members of the household and other members of the contact group. The index case and the source case were often the same person, but were not necessarily so. When several different cases of tuberculosis might have been the source of a contact's infection, no source case was specified for that contact.

In Edinburgh, following the notification of a case of tuberculosis, the Tuberculosis Health Visitor pays a visit to the patient's home. She makes a list of contacts of the patient, household and nonhousehold, and arranges appointments for all of them to attend the contact clinic at the Royal Victoria Dispensary. In this way a list of all important contacts was available, with basic information even for those who failed to attend. The majority, 91 per cent, of contacts of new cases were examined at the clinic at least once. On attendance, contacts up to and including twenty-five years of age were tuberculin tested by the Heaf multipuncture method (17). Any noteworthy symptoms were recorded. All contacts more than three years old had 70 mm. photofluorograms of the chest. In most cases the contacts were asked to come back at three-month intervals for the first year and at six-month intervals for the second year. After this point the situation was reviewed and contact supervision was discontinued unless there was any indication for further supervision. The contacts not followed up in this way were: (*1*) persons of more than fifty years of age in contact with patients with noninfectious sputum; (*2*) contacts of primary or nonrespiratory tuberculosis when all significant contacts had been examined (these two groups were discharged after one visit if healthy); (*3*) contacts with negative tuberculin tests were retested six weeks after all contact had been broken, and, if still negative, then were vaccinated with BCG vaccine. Six weeks after vaccination they had a further tuberculin test, and they were seen yearly thereafter for roentgenographic and tuberculin testing.

The present study is based on information about all new contacts for whom appointments were made at the Royal Victoria Dispensary between June 1, 1954, and May 31, 1955.

RESULTS

Only contact persons who attended the clinic for the first time during the study year were included in the survey. The majority of them were contacts of new cases, but a proportion were new contacts of old cases, e.g., babies born into tuberculous households. A total of 3,485 persons who were tuberculosis contacts were studied.

Age and Sex Composition of the Contact Population

The contact population in the survey by age and sex is shown in figure 1. The population of the city of Edinburgh (1951 census) is inset. There is a marked preponderance of young children in the contact population. This is largely due to the fact that many young children are brought to the contact clinic to receive BCG vaccination.

The household and nonhousehold contact groups are shown separately in fig-

ure 2. In figure 3 the population shown includes only the contacts of new cases, and the new contacts of old cases are excluded. For this purpose new cases of tuberculosis are defined as those diagnosed less than three months before the contact's first attendance.

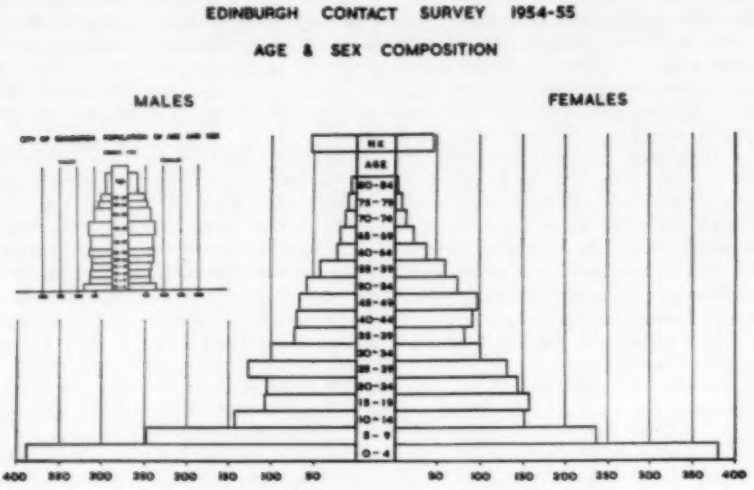

FIG. 1. Contact population: age and sex composition.

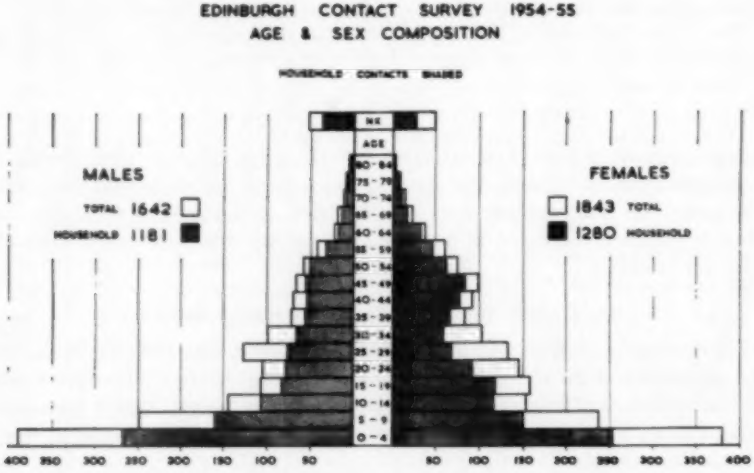

FIG. 2. Contact population, showing household contacts.

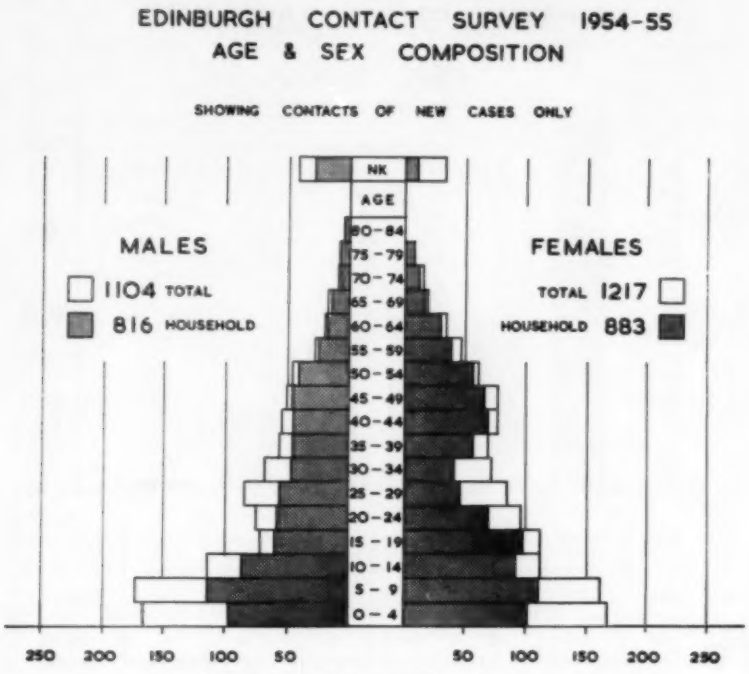

FIG. 3. Contact population: contacts of new cases.

Final Disposal

The disposal at the end of the first year's supervision of the 3,485 contacts is shown in figure 4. Of the 429 persons who did not attend the clinic, it has been verified that none were known to have tuberculosis a year after they should have first attended. It is known that 94 of them were in fact examined roentgenographically outside the contact clinic, with normal results.

The 1,202 contacts referred for BCG vaccination were followed up separately, and the results will be published elsewhere. None of the persons vaccinated who completed a year's observation were found to have developed tuberculosis.

Attendance and Default Rates

In order to assess the efficiency of contact examination in Edinburgh and to assess the validity of the findings in contacts, attendance and default rates were measured. The persons with tuberculosis contact who attended the contact clinic at least once within a year of being asked, or who attended unasked, were classed as "attenders." "Attenders" who were asked to return for follow-up examination

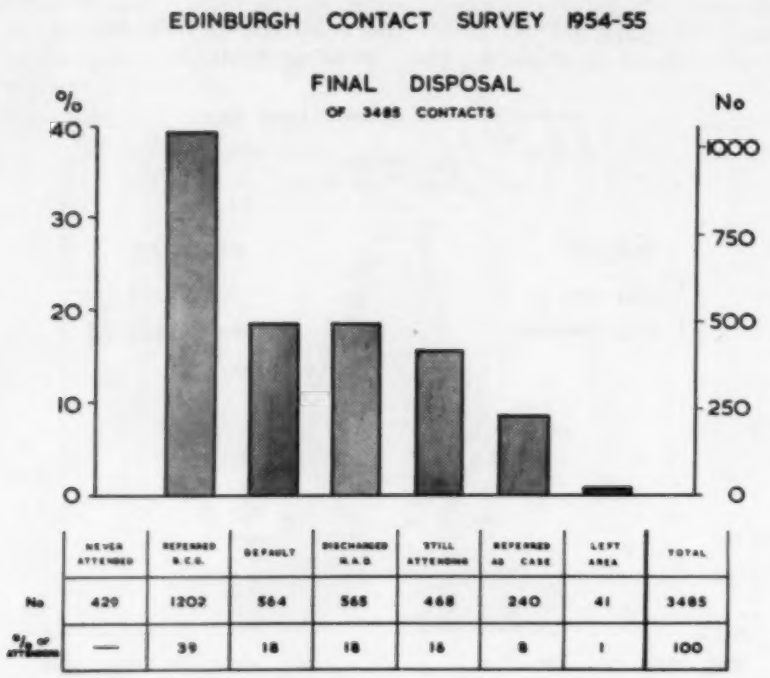

FIG. 4. Final disposal of contacts.

and failed to do so at any time during the year following their first attendance were classed as "defaulters."

The attendance rates for the household contact population are considered to be more accurate than those for the nonhousehold contact population because the household contacts were a more accurately defined population, and all household contacts were asked to attend. The nonhousehold population included a certain number of contacts who attended without being asked.

Of the total population of 3,485 contacts there were 429 "non-attenders" or 12.3 per cent (males 14.1 per cent and females 10.7 per cent). The attendance by age and sex is shown in table 1 and in figure 5.

The attendance rates for both sexes were high up to the age of fourteen years. From fifteen to twenty-four years of age attendance rates fell, being lower in males than in females. From twenty-five to forty years the rates improved in both sexes, being significantly better for females; beyond the age of forty years the rates fell off markedly for both sexes.

The attendance of household contacts varied little from that of the total contact population and was 87.5 per cent (males 85.4 per cent, females 89.4 per

TABLE 1

ATTENDANCE BY AGE AND SEX

Age Group	Male			Female			Both Sexes		
	Total Number	Non-Attendance	Per Cent Attendance	Total Number	Non-Attendance	Per Cent Attendance	Total Number	Non-Attendance	Per Cent Attendance
0–4	388	20	94.9	380	22	94.2	768	42	94.5
5–9	248	16	93.6	236	18	92.4	484	34	93.0
10–14	144	14	90.3	152	12	92.1	296	26	91.2
15–19	106	20	81.1	158	18	88.6	264	38	85.6
20–24	105	23	78.1	145	19	86.9	250	42	83.2
25–29	127	15	88.2	132	8	93.9	259	23	91.1
30–34	100	10	90.0	104	6	94.2	204	16	92.2
35–39	72	10	86.1	84	5	94.1	156	15	90.4
40–44	70	11	84.3	91	8	91.2	161	19	88.2
45–49	67	13	80.6	98	5	94.9	165	18	89.1
50–54	58	16	72.4	75	13	82.7	133	29	78.2
55–59	42	7	83.3	60	11	81.7	102	18	82.4
60–69	40	10	75.0	58	14	75.9	98	24	75.5
70–84	23	7	69.6	22	7	68.2	45	14	68.9
Not known	52	40	23.1	48	31	35.4	100	71	29.0
Total	1,642	232	85.9	1,843	197	89.3	3,485	429	87.7

cent). The age and sex distribution of attendance was very similar to that for the total population. Contacts of newly diagnosed cases (within three months before the date of the contact's first attendance) attended in almost the same proportion as the total contact population, with 88.6 per cent attendance (males 85.4 per cent, females 90.0 per cent).

It may be noted in table 2 that attendance rates were lower than average during the five months from November to March, inclusive, and higher than average from April to October, inclusive. "Non-attenders" were listed according to the month in which their first visit should have occurred. The non-attendance rate from April to October was 9.9 per cent, from November to March it was 15.5 per cent; this difference is significant ($P < 0.001$).

Other factors influencing attendance rates to a statistically significant degree are illustrated in figure 6. Contacts were grouped according to relationship to the index case as: (*1*) near relatives (parents, children, and siblings); (*2*) other relatives; and (*3*) not related to the index case. It will be seen that nonattendance was highest in unrelated, and lowest in closely related contacts, with other relatives occupying an intermediate position. The type of disease of the person who represented an index case also affected the attendance rates significantly. *Acute disease* was that judged on clinical and roentgenographic grounds to have been present for less than two years. *Chronic disease* was that judged to have been present for more than two years. The *chronic and acute* category included persons who had chronic disease with an acute exacerbation within the last two

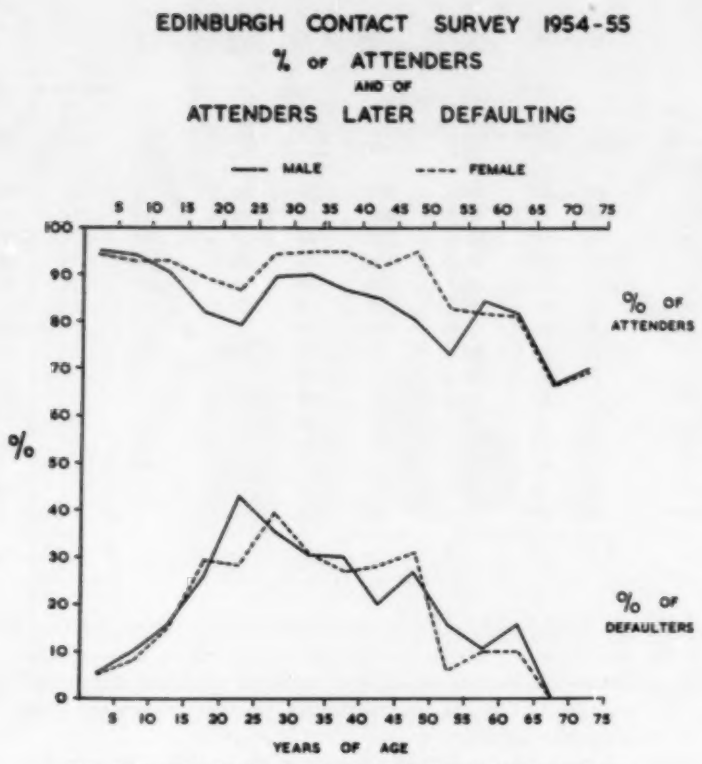

FIG. 5. Showing "attenders" and "defaulters" by age and sex.

years. Contacts of acute cases showed significantly better attendance rates than contacts of chronic cases, with or without an acute exacerbation. There was no difference between attendance rates of the contacts in the last two groups.

The cooperation of the person who represented an index case, judged by his willingness to submit to and continue supervision and treatment of his disease, was related to that of his contacts, as shown by attendance rates.

TABLE 2

"NON-ATTENDERS" MONTH BY MONTH

	January	February	March	April	May	June	July	August	September	October	November	December	Total
Contacts	270	317	386	320	268	388	341	232	181	293	349	212	3485
Non-attenders	43	45	66	30	28	46	34	21	20	22	44	30	429
Per cent non-attenders	15.4	14.2	21.6	9.4	10.5	11.9	10.0	9.1	11.0	7.5	12.6	14.2	12.3

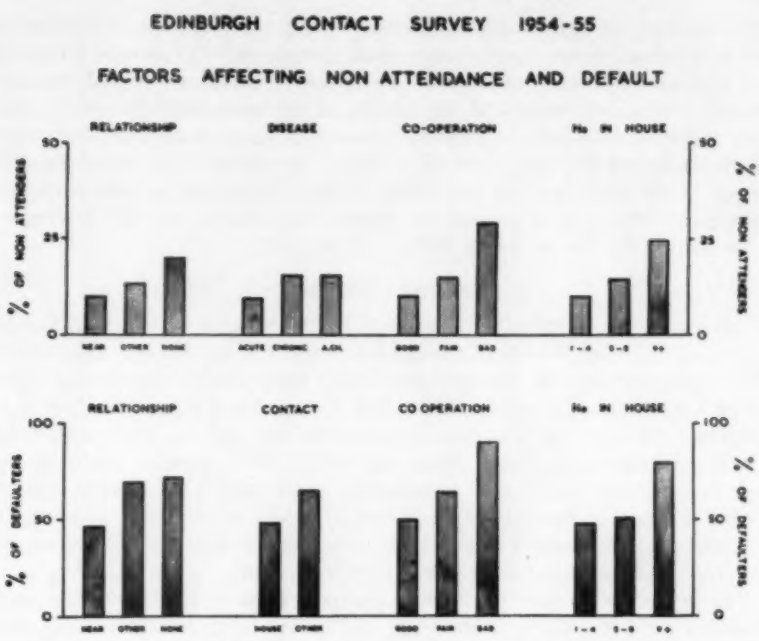

FIG. 6. (Upper) Showing factors having a significant effect on attendance rates.
FIG. 7. (Lower) Showing factors having a significant effect on default rates.

The number of persons in the household of the index case affected the attendance of the contacts, as shown in figure 6. It may be seen that when there was a large number in the household attendance was less complete.

The extent of disease (minimal, moderate, or far advanced, according to National Tuberculosis Association standards) of the index case had no apparent effect on the attendance of the patient's contacts.

Factors Influencing Default from Follow-up Appointments

In figure 5 is shown the age and sex distribution of "attenders" who later defaulted follow-up appointments during the first year, expressed as a percentage of the total number of "attenders" in their age-sex group. The persons who were tuberculin negative and were referred to the BCG Clinic and persons more than fifty years old who were not asked to return for follow-up examination did not have an opportunity to default.

Other factors affecting the default rates are shown in figure 7. Here, the defaulters are expressed as a percentage of the number of contacts who were asked to attend for follow-up contact examination throughout the year.

As with "non-attenders," relationship to the person representing the index case, cooperation of the index case, and the number in the household of the in-

dex case were all significantly associated with the default rate. Nonhousehold contacts defaulted significantly more often than household contacts. Household and nonhousehold contacts could not be compared satisfactorily with regard to original attendance because of the loading of the nonhousehold contact group with uninvited attenders. All differences shown in figures 6 and 7 are statistically significant by the chi-square test ($P < 0.01$). The chronicity or acuteness of the disease in the index case did not appear to have as marked an association with subsequent default as it had on the original attendance, and the difference is more likely to be due to chance ($0.05 > P > 0.02$).

Tuberculin Test Results

Tuberculin tests were carried out on persons who were tuberculosis contacts up to and including the age of twenty-five years. The routine test used was the Heaf multipuncture test, but in 41 instances a Mantoux test was done, using 0.1 ml. of 1:1,000 Old Tuberculin (OT), with a second test using 0.1 ml. of 1:100 OT when the first test was negative. Four of the persons who were tuberculosis contacts refused any tuberculin test. The tuberculin reactions were read between forty-eight and seventy-two hours, and 1,810 results were recorded. Forty-four contacts (2.4 per cent of those tested) failed to return for test reading at the correct time. There were positive results in 636 cases (35.1 per cent), and negative results in 1,174 (64.9 per cent).

The persons who were tuberculosis contacts were grouped according to the bacteriologic status of the source case, four groups being established as follows:

(*1*) Those in contact with a person whose sputum was at any time positive for tubercle bacilli on microscopy.

(*2*) Those in contact with a person whose sputum was always negative on microscopy, but was at any time positive for tubercle bacilli on culture.

(*3*) Those in contact with a person without sputum whose gastric lavage or laryngeal swab was at any time positive for tubercle bacilli on culture.

(*4*) Those in contact with a person whose sputum, gastric lavage, or laryngeal swab was tested and always found negative for tubercle bacilli.

Percentages of household contacts in the different bacteriologic groups found to be tuberculin positive are shown in table 3 and figure 8. The numerically smallest groups of contacts were *Groups 2* and *3*. These two groups showed little difference in the percentage positive to tuberculin and have been combined in the right-hand portion of figure 8. The differences which are statistically significant by the chi-square test are indicated. The age distribution of contacts in the different groups was examined and did not vary in the groups to any significant extent. The same comparison of the proportion who were tuberculin positive in the different bacteriologic groups is shown by age group in figure 9.

Household and nonhousehold contacts are compared in figure 10. The general level of infection was lower in the nonhousehold group, both for the contacts as a whole and for each age group.

Consideration was given to the strength of the tuberculin reaction in the contacts tested. The percentage of household and nonhousehold contacts with weak or strong tuberculin reactions in the different bacteriologic groups is shown

TABLE 3

TOTAL NUMBER OF HOUSEHOLD CONTACTS WHO WERE TUBERCULIN TESTED, IN AGE GROUPS AND ACCORDING TO BACTERIOLOGIC STATUS OF SOURCE CASE

	0–4			5–9			10–14			15–19			20–25			0–25		
	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive
Never positive for tubercle bacilli	159	20	12.6	112	14	12.5	76	27	35.5	57	34	59.7	44	33	75.0	448	128	28.6
L.S./G.L.* positive for tubercle bacilli	44	12	27.3	26	8	30.8	17	9	52.9	10	5	50.0	5	4	80.0	102	38	37.3
Culture positive for tubercle bacilli	56	10	17.9	33	9	27.3	20	11	55.0	20	13	65.0	23	19	82.6	152	62	40.8
L.S./G.L. and culture positive for tubercle bacilli	100	22	22.0	59	17	28.8	37	20	54.1	30	18	60.0	28	23	82.1	254	100	39.4
On microscopy positive for tubercle bacilli	127	40	31.5	64	41	64.1	35	25	72.4	42	35	83.3	35	32	91.4	303	173	57.1
Total	386	82	21.2	235	72	30.6	148	72	48.7	129	87	67.4	107	88	82.2	1,005	401	39.9

* L.S.—laryngeal swab.
G.L.—gastric lavage.

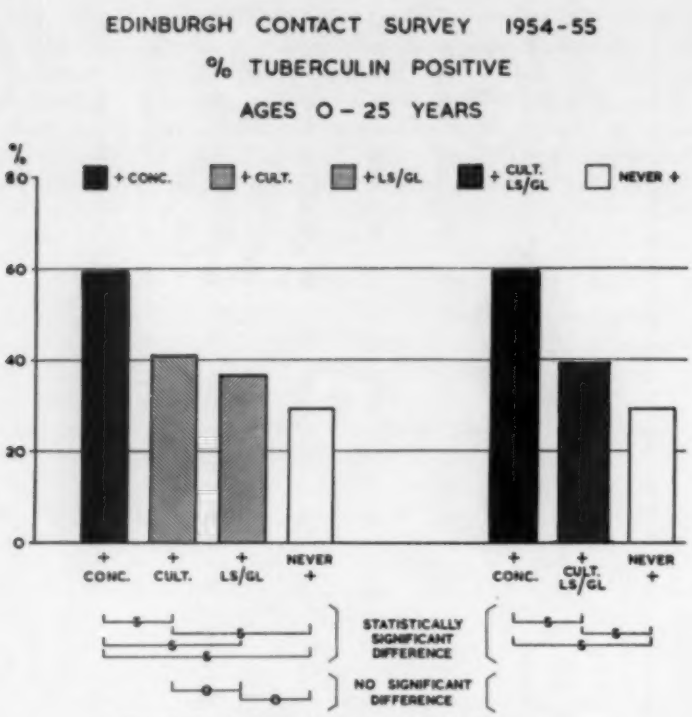

FIG. 8. Contact population: contacts of new cases.

in figure 11. The Heaf test results were graded as negative; positive grade *1*, grade *2*, grade *3*, and grade *4*, as described by Heaf (17). Positive grades *1* and *2* have been combined here as "weakly positive," and positive grades *3* and *4* have been combined as "strongly positive." In the few cases in which the Mantoux test was used, induration of 10 mm. or more following testing with 0.1 ml. of 1:1,000 OT was classed as "strongly positive"; and less than 10 mm. of induration was classed as "weakly positive" when more than 10 mm. of induration was obtained on subsequent testing with 0.1 ml. of 1:100 OT. The dividing lines between "weakly" and "strongly" positive in the two different tests used are believed to correspond reasonably well. It will be seen that the higher grades of tuberculin sensitivity were found in the groups with the highest proportion of tuberculin-positive contacts. The difference in the grade of tuberculin sensitivity is significant only in the case of the household contacts ($0.02 > P > 0.01$). Thus, it appears that the contacts in the groups most likely to be infected were also those most likely to give strongly positive reactions to tuberculin.

Contacts Referred for Further Investigation

Contacts were referred for further clinical investigation whenever the roentgenogram showed a significant abnormality, when tuberculin "conversion" was

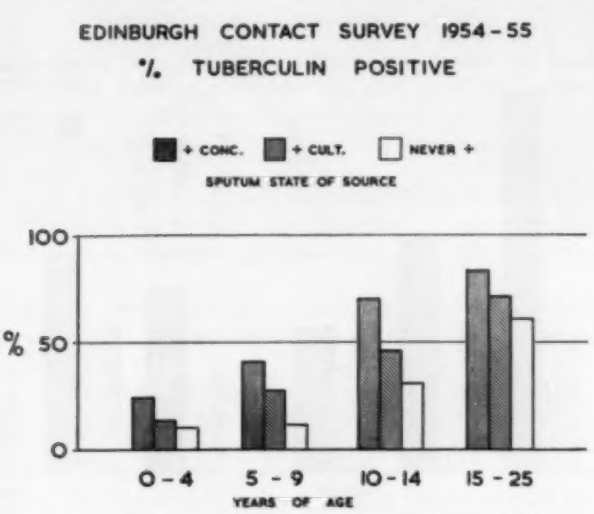

FIG. 9. Showing percentage of contacts who were tuberculin positive in age groups and by bacteriologic status of source case.

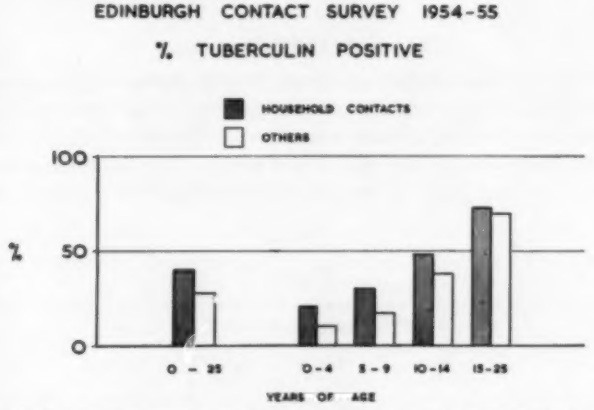

FIG. 10. Showing percentage of contacts who were tuberculin positive in household and nonhousehold contact groups; total and in age groups.

observed, and when a child of three years old or less was found to have a positive tuberculin reaction.

Of the 3,056 contacts who attended the clinic, 240 (7.9 per cent) were referred for further investigation. No abnormality was found in 21 of these, their referral being due to "over-reading" of 70 mm. films, artifacts, doubt about tuberculin conversion, and similar factors.

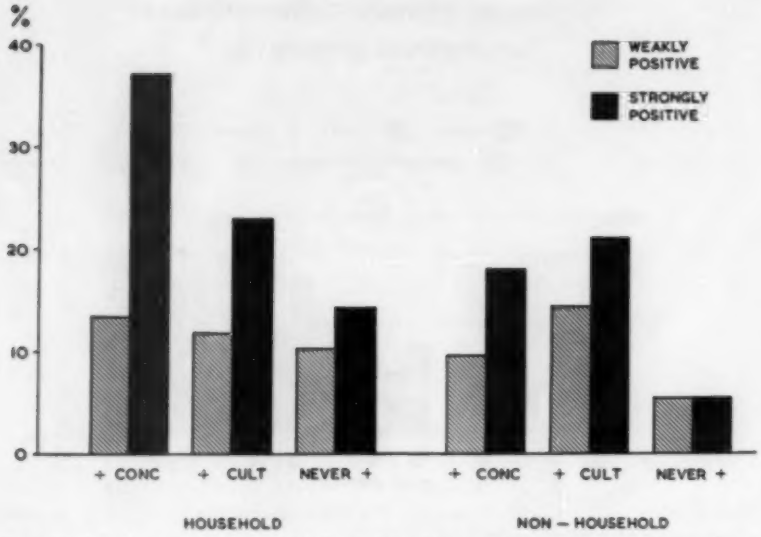

FIG. 11. Showing percentage of household and nonhousehold contacts weakly and strongly positive to tuberculin, grouped according to bacteriologic status of source case.

Tuberculosis Among Contacts

Thirty contacts were referred as tuberculin "converters"; 24 because they had positive tuberculin tests when less than three years old; 115 were considered after a year's observation to have respiratory tuberculosis of significant degree; and 39 were considered at the end of the year to have insignificant respiratory tuberculosis (calcified primary complexes, et cetera). These groups are shown in table 4.

TABLE 4

CONTACTS REFERRED FOR CLINICAL EXAMINATION AND FOUND TO HAVE TUBERCULOUS CONDITIONS

	Significant Respiratory Tuberculosis	Insignificant Respiratory Tuberculosis	Tuberculin Converters	Positive Tuberculin Test $\leq$ 3 years
Male	58	20	15	16
Female	57	19	15	8
Total	115	39	30*	24
Per cent of all attenders	3.8	1.3	1.0	0.8

* The 30 tuberculin converters include 10 with lesions who are also in the significant respiratory tuberculosis group.

Significant Respiratory Tuberculosis

This group includes the tuberculosis contact persons who had lesions which, after a year's clinical observation, were considered to be tuberculous in origin and to require either treatment or continued observation. There were 115 persons in this group, 58 males and 57 females. This represented 3.8 per cent of all of the "attenders."

Ninety-seven (4.5 per cent) of the 2,154 household contacts and 18 (2.0 per cent) of the 903 nonhousehold contacts had significant respiratory tuberculosis. The difference between these groups might have been due to chance ($0.05 > P > 0.01$).

The prevalence by age and sex of significant respiratory tuberculosis is shown in table 5, as well as the prevalence among those tested and found to be positive to tuberculin.

The 115 cases comprised 59 post-primary cases, 50 primary, and 6 cases of pleural effusion. There was no sex difference in the distribution of types of disease. The extent of disease was minimal pulmonary tuberculosis in 67 cases, moderately advanced in 36 cases, far advanced in 6 cases, and pleural effusion in 6 cases. Cavitation was present in 7 males and 3 females; 21 of these patients had sputum or laryngeal swabs positive for tubercle bacilli, and in 94 the specimens were negative on examination. Fifteen of the 115 persons reported symptoms at the time of contact examination. Cough, sputum, and "recent cold" were the most common complaints.

Disposal

Forty-nine of the contacts found to have significant respiratory tuberculosis were admitted to the hospital. Five were given bed rest at home with chemotherapy, and 20 had chemotherapy while ambulant. Forty-one of the contacts had observation only, with no treatment during the year following first attendance as contacts of patients with tuberculosis.

TABLE 5

CONTACTS WITH SIGNIFICANT RESPIRATORY TUBERCULOSIS IN AGE AND SEX GROUPS, AND ALSO THE TOTAL NUMBERS IN THESE GROUPS

Age	Number of Contacts Attending			Number with Significant Respiratory Tuberculosis			Percentage with Significant Respiratory Tuberculosis			Total Number in Age Group with Positive Tuberculin Reaction			Percentage of Positive Tuberculin Reactors with Significant Respiratory Tuberculosis		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
0–4	369	358	727	12	7	19	3.3	2.0	2.6	73	45	118	16.4	15.6	16.1
5–9	232	218	450	10	12	22	4.3	5.5	4.9	50	53	103	20.0	22.6	21.4
10–14	130	140	270	5	3	8	3.9	2.1	3.0	54	54	108	9.3	5.6	7.4
15–19	86	140	226	5	5	10	5.8	3.6	4.4	52	83	135	9.6	6.0	7.4
20–29	195	253	448	6	7	13	3.1	2.8	2.9	67	95	162	9.0	7.4	8.0
30–39	154	187	341	10	6	16	6.5	3.2	4.7						
40–49	114	177	291	4	11	15	3.5	6.2	5.2						
50+	131	173	304	6	6	12	4.6	3.5	4.0						
Total	1,411	1,646	3,057	58	57	115	4.1	3.5	3.8						

TABLE 6

NUMBERS OF CONTACTS WITH SIGNIFICANT RESPIRATORY TUBERCULOSIS IN THE DIFFERENT RELATIONSHIP GROUPS TO THEIR INDEX CASES AND TOTALS IN EACH RELATIONSHIP GROUP

	Total Number of Attenders in Relationship Group	Number of Contacts with Significant Respiratory Tuberculosis	Percentage with Significant Respiratory Tuberculosis
Spouses	322	16	5.0
Siblings	500	23	4.6
Offspring	744	34	4.6
Parents	366	18	4.9
Other relatives	414	11	2.7
Not related	711	13	1.8

Relationship to Index Case

The number of "attenders" in each relationship group and the percentage with significant respiratory tuberculosis may be seen in table 6. The difference in the prevalence in the relationship groups could be due to chance ($0.05 > P > 0.01$). If only the related groups are compared with the unrelated groups, the difference is statistically significant ($P < 0.01$).

Time of Referral for Further Investigation

Of the 115 contacts referred for clinical examination and found to have significant respiratory tuberculosis, 91 persons were referred at their first visit; 7 were referred six weeks later; 8, three months later; 4, six months later; 2, nine months later; and 3, twelve months from the time of the first contact examination. Thus 24 contacts were referred after a follow-up visit. Of these, 10 were tuberculin "converters" and would have been referred for this reason, even if the chest roentgenograms had not shown lesions. Seven of the remaining 14 persons were found in retrospect to have had lesions present at the first visit, and these were either overlooked by the observers or were seen and classed as insignificant. Hence, only 7 of the 115 persons (6.1 per cent) were dependent on follow-up roentgenograms for their referral for further investigation. Five of the 7 had pleural effusions, 4 with symptoms at the time of diagnosis. Four were referred six months after the first examination, and one at three months. The other 2 cases that developed after the first visit included a male of twenty-four years who was referred at three months with moderately advanced cavitary tuberculosis, having had a normal roentgenogram at the time of the first visit. He was a contact of his father, who had highly infectious sputum. This twenty-four-year-old male had definite symptoms of the disease three months after the first examination as a contact of his father. The other case was that of a female, thirty-four years of age, who was referred at twelve months with a minimal lesion not present at previous visits. She had nursed her father-in-law during his last illness. The father-in-law was reported after death as tuberculous and presumably had been infectious.

Of the 115 contacts with significant respiratory tuberculosis, the source case was known in 77 cases. These source cases have been grouped by bacteriologic

TABLE 7

SPUTUM STATUS OF SOURCE (WHEN KNOWN) FOR CONTACTS WITH SIGNIFICANT RESPIRATORY TUBERCULOSIS OR WHO WERE TUBERCULIN CONVERTERS

Sputum State of Source	Significant Respiratory Tuberculosis	Converters Without Lesion	Total
Microscopy positive for tubercle bacilli	53	8	61
Culture positive for tubercle bacilli	5	3	8
L.S./G.L.* positive for tubercle bacilli	13	0	13
Negative for tubercle bacilli	3	1	4
Death notification	3	0	3
Total	77	12	89

* L.S.—laryngeal swab.
G.L.—gastric lavage.

status and are shown in table 7. The 12 contacts who underwent tuberculin "conversion" (without lesion) and whose source cases were known are also included in this table.

It may be seen from table 7 that 13 tuberculosis contacts who developed significant respiratory tuberculosis had, as their source of disease, patients with demonstrable tubercle bacilli on laryngeal swab or gastric lavage. All of these "source" patients had disease of considerable extent, some with cavitation. Most of them represented cases in which the absence of sputum was a surprising feature and was probably a result of the patient's failure to realize or admit its existence.

The three contacts with significant respiratory tuberculosis and the one tuberculin "converter," who had as their source a patient with noninfectious sputum, were contacts of patients with newly diagnosed disease with whom close contact had occurred. The initial sputum tests were negative for tubercle bacilli and treatment was instituted before further tests were done.

Nonrespiratory Tuberculous Lesions

One girl, one year old, had tuberculosis of the spine in addition to a primary complex in the lung. Her mother had tuberculosis with highly infectious sputum. One three-year-old boy was tuberculin negative at the first visit; six weeks later he "converted" and had a primary lung complex, and very shortly afterwards developed tuberculous meningitis. He was in contact with his father, who had pulmonary tuberculosis with highly infectious sputum.

Insignificant Respiratory Tuberculosis

Thirty-five contacts showed lesions which were finally classed as insignificant respiratory tuberculosis. None of the persons was less than fifteen years old and most were twenty-five to thirty-four years of age. None showed signs of activity of their lesions during the year's observation.

Tuberculin "Converters"

Thirty contacts were seen to "convert" from negative to positive reactors to tuberculin. This represented 1.0 per cent of all contacts, or 1.7 per cent of con-

tacts on whom tests were done. Ten of them had tuberculous lesions at the time of the second test, and they have been included in the significant respiratory tuberculosis group. The remaining 20 consisted of 9 males and 11 females. Four had symptoms, 3 complaining of a "recent cold" and one of listlessness. None developed tuberculous lesions during the year after their first visit.

Contacts of Three Years of Age or Less with Positive Tuberculin Tests

There were 24 such children. They were supervised carefully; none developed disease during the year of observation.

Other Diseases

Twenty-three contacts were found to have nontuberculous chest disease of note, including chronic bronchitis and emphysema, bronchiectasis, pneumonia, pneumoconiosis, tumors, and other diseases.

Discussion

It is believed that a high proportion of persons who are tuberculosis contacts can be examined at least once by using the routine methods of persuasion by health visitors and by provision of adequate facilities. Evening sessions at the chest clinic for the examination of contacts well repaid the extra work involved. In this survey 88 per cent of all invited contacts attended the clinic.

The factors influencing contact attendance have been studied, and it is shown that age is important in this respect. Attendance was poor between the ages of fifteen and twenty-five, and by those more than forty. The attendance was worse, generally, in males than in females; between the ages of twenty-five and forty years, the sex difference in attendance was more than would be expected to result from chance ($P < 0.01$).

The seasonal variation in attendance rates was surprisingly large, and it is difficult to know what can be done to remedy this irregularity. The poorer attendance among contacts who are members of large households is presumably due to the difficulties of organizing the visit of a large brood of children and to higher transportation costs.

The better attendance rates among closer relatives of the person who represented an index case and the poorer attendance among contacts of uncooperative patients are perhaps to be expected. Contacts of patients suffering from acute disease attend better than contacts of patients with chronic disease. This may be due to the more forcible impact of the acute disease on the minds of the contacts, with its increased likelihood of symptoms. The same factors seem to play a part in determining whether contacts will continue to attend for supervision or will default.

With respect to infection rates, the level of tuberculous infection was higher in contacts than in noncontacts. From the report of the Medical Officer of Health for Edinburgh for 1955, it may be seen that 24.3 per cent of school children aged thirteen to fourteen years were tuberculin positive by the Heaf test. For contacts aged thirteen to fourteen years, the percentage positive was 47.6.

The proportion of infected contacts increases with the profusion of tubercle bacilli in the sputum of the source of infection. The infection rate varied from

25.5 per cent when the patient representing the source case had sputum negative for tubercle bacilli to 46.8 per cent when the sputum was positive on microscopy. The tuberculin reaction was positive in 38.4 per cent of contacts of patients with sputum, laryngeal swab, or gastric lavage positive for tubercle bacilli on culture.

The importance of these findings is obvious in that for the patient with sputum, laryngeal swab, or gastric lavage positive for tubercle bacilli, any test must be regarded as a possible public health risk. It is important to emphasize that persons in whom tubercle bacilli are demonstrated by laryngeal swab or gastric lavage may be infectious when lung destruction has taken place and may have quite a different significance from the same bacteriologic findings in the case of primary tuberculosis without lung destruction and without cough or sputum. Household contacts show a rather higher level of infection than nonhousehold contacts. This finding applies to all age groups and is not due to the different age composition of the household and nonhousehold contact groups.

An interesting and unexpected finding is that the degree of tuberculin sensitivity in contacts varied with the sputum status of the source case. When the sputum of the patient representing the source case was positive for tubercle bacilli on microscopy, a higher proportion of contacts positive to tuberculin were strongly positive than when the source sputum was positive only on culture or was negative on all tests.

Of the 115 contacts with significant respiratory tuberculosis, only seven new lesions appeared later than the first visit among contacts who were tuberculin positive at the first visit. Five of these seven were cases of pleural effusion, all detected within six months. The other 2 were cases of acute disease in contacts who had been exposed to heavy infection.

While no annual attack rate for the whole population who attended the clinic originally can be calculated because of the default rate, it seems likely that respiratory tuberculosis in contacts is now most likely to be detected at first attendance. It may be that follow-up examination is worth while only in those contacts who have been exposed to heavy infection. It seems likely that, when resources are limited, it would be better to use them to obtain the highest possible attendance of contacts at least once rather than to dissipate them in less profitable follow-up work.

In 68.8 per cent of contacts with significant respiratory tuberculosis in whom the source of infection was known, the sputum of the "source" patient was positive for tubercle bacilli on microscopy. Patients with sputum, laryngeal swab, or gastric lavage positive for tubercle bacilli on culture accounted for 6.5 per cent and 16.9 per cent, respectively, of the tuberculosis contacts who developed significant respiratory tuberculosis. It is important to remember that patients whose sputum is positive for tubercle bacilli only on culture are nevertheless infectious, and contact with them may result in the contraction of tuberculosis.

Summary

A survey of 3,485 persons who were contacts of tuberculous patients was carried out in Edinburgh in 1954–1955; 88 per cent or 3,056 contacts were studied. Of the contacts who were tuberculin tested, 25.5 per cent were positive when the "source" patient's sputum, laryngeal swab, or gastric contents were negative

for tubercle bacilli on culture; 38.4 per cent were tuberculin positive when the "source" patient's sputum, laryngeal swab, or gastric contents were positive for tubercle bacilli on culture only; and 48.8 per cent were tuberculin positive when the "source" patient's sputum was positive on microscopy. There is a statistically significant difference in the infectiousness of the three source case groups.

Of the 3,056 contacts examined, 115 (3.8 per cent) showed significant respiratory tuberculosis. Of these 115, only 7 developed the lesions at a time subsequent to the first visit, having been tuberculin positive at the first visit; 6 developed their lesions within six months of the first visit.

It is important to try to examine all contacts of a patient with tuberculosis at least once, but the value of follow-up examination is doubtful except in the susceptible age groups when the exposure to infection is heavy. When facilities are limited, it is probably more profitable to use them to obtain high rates of initial attendance of contacts rather than to spend them on less fruitful follow-up studies.

Sumario

Análisis de 3,485 Casos de Contacto con la Tuberculosis, Edimburgo, 1954–1955

En Edimburgo se llevó a cabo un estudio de 3,485 casos de contacto con la tuberculosis en 1954–1955. A 3,056 (88 por ciento) se les examinó a lo menos una vez. Se estudiaron los factores que afectan la falta de asistencia a la clínica para contactos. Un 25.5 por ciento de los sujetos que habían estado en contacto con tuberculosis y fueron comprobados con tuberculina resultó positivo cuando el foco era negativo bacteriológicamenta; un 38.4 por ciento resultó positivo a la tuberculina cuando el foco era positivo al cultivo del esputo, del frote laríngeo o del contenido gástrico; un 48.8 por ciento resultó positivo a la tuberculina cuando el foco producía esputo positivo para bacilos tuberculosos al estudiarlo microscópicamente. Hay una diferencia de importancia estadística en la infecciosidad de los tres grupos de casos vinculados con distintos focos.

Ciento quince (3.8 por ciento) de los casos de contacto examinados revelaron tuberculosis importante del aparato respiratorio. Sólo 7 de los 115 manifestaron sus lesiones después de la primera visita, habiendo ya sido en ésta positivos a la tuberculina; 6 de ellos manifestaron sus lesiones en término de seis meses de la primera visita. Es importante tratar de examinar a todos los casos de contacto a lo menos una vez, pero es dudoso el valor de un examen subsecuente excepto en los grupos de edad susceptible cuando es intensa la exposición a la infección. Cuando los medios son limitados, resulta probablemente más provechoso utilizarlos para conseguir proporciones altas de asistencia inicial de los casos de contacto que dedicarlos a menos útiles estudios de observación subsiguiente.

Resume

Analyse concernant 3.485 sujets en contact avec la tuberculose, Edimbourg 1954–1955

Une enquête concernant 3.485 sujets en contact avec l'infection tuberculeuse a été effectuée à Edimbourg en 1954–1955. Parmi ces sujets, 3.056 ont été examinés au minimum une fois. Les facteurs prévenant les visites à la clinique affectée à l'enquête furent étudiés. Vingt-cinq et cinq dixièmes pour cent de ces sujets avaient une réaction positive à la tuberculine quand le contage était une tuberculose bactériologiquement négative; 38,4 pour cent avaient une réaction positive à la tuberculine quand le contage était une tuberculose avec expectoration, prélèvement laryngé ou lavage gastrique positifs en culture; 48,8 pour cent avaient une réaction positive à la tuberculine quand le contage était une tuberculose à expectoration positive à l'examen direct. La différence dans l'infectiosité des trois groupes, selon le contage, était statistiquement significative.

Cent-quinze (3,8%) des sujets en contact avec la tuberculose présentaient une tubercu-

lose du système respiratoire statistiquement significative. Parmi ces 115 sujets 7 seulement ont présenté des lésions après la première visite, mais leur réaction à la tuberculine était positive à la première visite; chez 6 d'entre eux les lésions se sont développées dans les six mois qui suivirent la première visite. Il est important d'essayer d'examiner au moins une fois tous les sujets en contact avec la tuberculose, mais l'utilité d'examens périodiques est douteuse, sauf dans les groupes d'âge sensible à l'infection quand l'exposition à la tuberculose est importante. Quand on dispose de moyens limités, il est probablement plus avantageux de les utiliser pour obtenir un taux élevé de présence à l'examen initial des sujets en contact avec la tuberculose, plutôt que de les gaspiller pour la mise en observation ultérieure des sujets, ce qui est moins utile.

Acknowledgments

Many people contributed directly and indirectly to this survey. In particular, thanks are due to Dr. L. Stein and Mr. S. A. Sklaroff of the Department of Social Medicine, Edinburgh University, for much assistance in planning the survey and guidance in carrying it out. It is a pleasure for the writers to record their appreciation of the work of the Tuberculosis Health Visitors of the City of Edinburgh, whose efforts have achieved such satisfactory attendances at the contact clinic. Dr. Ian C. Brannen, formerly of this Department, is also to be thanked for his assistance in coding information and in the routine work. The physicians attending the Royal Victoria Dispensary kindly supplied information about the cases of tuberculosis under their care and allowed full access to their clinical records.

To the Royal Victoria Trust the thanks of the writers are due for defraying part of the cost of this investigation.

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THE COALESCENT LESION OF DIATOMACEOUS EARTH PNEUMOCONIOSIS[1]

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Introduction

Although Legge and Rosencrantz (1) did not describe the appearance of the lesions they saw on the chest roentgenograms of diatomaceous earth workers, it was assumed by most readers that they were referring to typical lesions of nodular silicosis. It remained for Smart and Anderson (2) to show conclusively that the inhalation of diatomaceous earth and its processed products produces a distinct and different type of reaction than does quartz. At no time did they observe nodulation in the roentgenographic changes of the workers, and it was this initial observation which stimulated them to classify the basic pathologic and roentgenographic lesions of diatomaceous earth pneumoconiosis. Smart and Anderson point out that diatomaceous earth differs from quartz in that it is an amorphous hydrated silica and exists in the form of an opal. Although there is a difference in the toxicity of the various processed products in the diatomaceous earth industry, as well as in the marine and fresh water varieties, the majority of the workers with lung changes were exposed to many or all of the different products. Admitting, therefore, the complexity of any abnormal lung changes observed, they nevertheless did present for future observers a workable roentgenographic classification. Briefly, the workers who have developed pulmonary roentgenographic changes fall into one of two types: those having pneumoconiosis of linear type; and those with coalescent or massive lesions.

Smart and Anderson (2) take a firm stand in their conviction that infection plays a small part, if any, in the production of the coalescent lesions observed in the course of the disease. In this they admit their disagreement with Pendergrass (3) who implies that coalescence in pneumoconiosis usually means associated infection, either concomitant or healed. It is in the coalescent lesion of this disease that the present writer is primarily interested.

This report was written after a ten-year period of observation and follow-up study of a small group of patients with diatomaceous earth pneumoconiosis and coexisting active pulmonary tuberculosis. Brief case histories, chest roentgenograms, and the pathologic findings in 4 patients with proved active pulmonary tuberculosis and diatomaceous earth pneumoconiosis are presented to support a hypothesis that both diseases played a part in the pathogenesis of their coalescent lesions. Second, the case histories and chest roentgenograms of 2 patients, who have not had pathologic lung studies, are presented because it is believed that sufficient evidence is present to recommend the use of anti-

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microbial therapy in any patient with a history of exposure to diatomaceous earth dust, pulmonary roentgenographic changes, and a positive tuberculin reaction. Finally, the case histories and chest films of 2 patients with diatomaceous earth pneumoconiosis and consistently negative reactions to tuberculin are presented to demonstrate a significant difference in the roentgenographic appearance of their coalescent lesions when compared with those of proved active tuberculosis and coexisting diatomaceous earth pneumoconiosis.

Case Reports

Case 1. The patient (No. 20 625), a white male, born in 1911, had been employed in the diatomite industry from 1933 until 1942. A chest roentgenogram obtained on November 9, 1936, was normal. A chest film obtained on June 20, 1939, after he had been in the industry for three years, showed typical bilateral linear lesions of diatomaceous earth pneumoconiosis with beginning coalescence in the right upper lobe. In March, 1946, the patient was admitted to the Pulmonary Disease Service of the Santa Barbara General Hospital with a diagnosis of active pulmonary tuberculosis confirmed by sputum positive for tubercle bacilli on microscopy and culture. A chest roentgenogram taken on admission, March 30, 1946 (figure 1) showed a tuberculous cavity with a fluid level in the right apex. The coalescent lesion in the left upper lobe resembled tuberculosis, but was indistinguishable from some of the coalescent lesions described by Smart and Anderson (2) as uncomplicated by infection. The patient was discharged from the hospital in June, 1948, with presumably inactive disease and got along fairly well until March, 1949, when he was readmitted be-

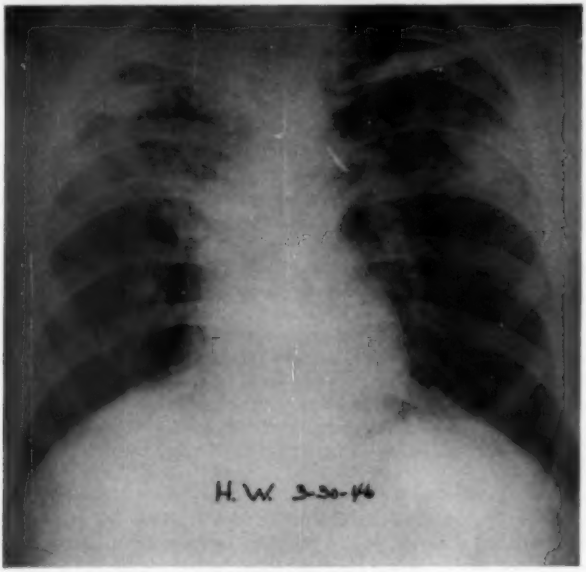

Fig. 1 (No. 20 625). Hospital admission film obtained on March 30, 1946. The tuberculous cavity with fluid level in the right first interspace is demonstrated. Particular interest in this case is directed to the large coalescent lesion in the middle third of the left lung resembling tuberculosis.

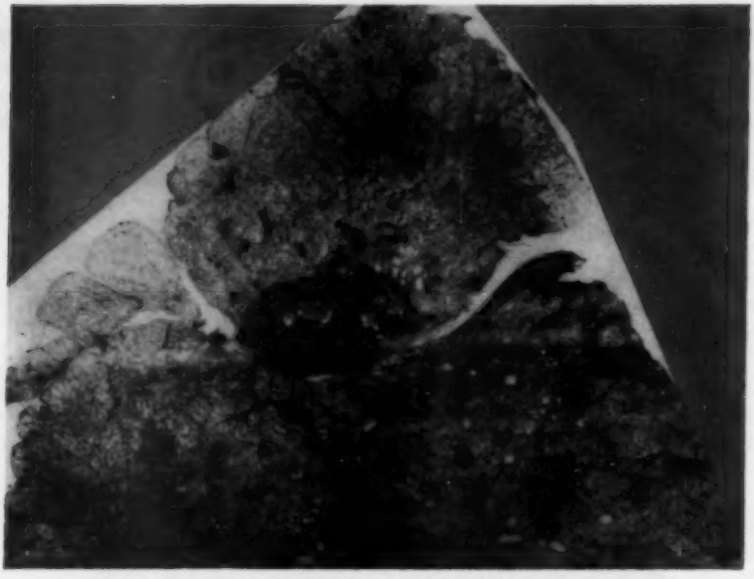

FIG. 2 (No. 20 625). Sagittal section of the upper half of the left lung, showing coalescent fibrocaseous lesions in the base of the upper lobe and in the adjoining segment of the lower lobe, with satellite small fibrocaseous nodules. Note the lack of emphysema.

cause of the reappearance of the right upper lobe cavity and sputum positive for tubercle bacilli. From this point on, his course was steadily downhill and he developed signs of cor pulmonale. A chest film taken on September 22, 1953, revealed an apparent tuberculous pneumonia in the left lower lobe and contraction of the coalescent lesion in the left second interspace. In spite of antimicrobial and supportive therapy, he developed Cheyne-Stokes respirations and died on October 7, 1953.

An autopsy was performed and a portion of the pathologic report is as follows:

> "There are two separate pulmonary diseases, tuberculosis and pneumoconiosis, most of the lesions of which are distinctly identifiable and separate, one from the other." . . . "Two features of the tuberculosis are unusual, viz., (*1*) the large 'tuberculomas' of the left upper and lower lobes, and (*2*) the infection of the hilar lymph nodes. The 'tuberculomas' are essentially large areas of caseous pneumonia which have become circumscribed and are undergoing healing and encapsulation. They are unusual in being so large and in their rather markedly fibrous consistency. They are also unusual in location, occupying adjoining segments of two lobes without perforating the interlobar pleura. . . .
>
> "It is pertinent to consider in what way, if any, the pulmonary tuberculosis and pneumoconiosis are interrelated. This question cannot be answered with finality by the pathological examination of the patient's lungs alone. Clinical, epidemiological, and experimental data will provide more important evidence in the long run. There is no evidence from the pathological examination that the diffuse reticular fibrosis has been modified by the infection. There is reason to suspect, however, that the excessive localized fibrosis, represented by the tuberculomas in the left lung, may have been conditioned in part by the pneumoconiosis. This suspicion is based upon the published

experiences of other workers with the effect of 'inert' dusts on the healing of tuberculous lesion in animals' lungs and on the prevailing concept of the combined effect of silica and tuberculosis in the production of conglomerate fibrosis in human lungs."

The photograph of a sagittal section of the upper half of the left lung (figure 2) was made, showing the extensive combined tuberculous and pneumoconiotic lesions. The large fibrocaseous lesion at the base of the upper lobe undoubtedly produced the conglomerate density in the left upper lobe in figure 1.

Comment: An attempt has been made in this case to correlate the clinical, roentgenographic, and pathologic findings with particular reference to the coalescent fibrocaseous lesions in the base of the upper lobe and adjoining segment of the lower lobe. No attempt is made at this time to consider the effect the pneumoconiosis may have had on the unusual clinical course of the superimposed tuberculosis. The presence of two separate diseases in this patient's lungs has been proved, and attention has been called to the probability that both tuberculosis and diatomaceous earth pneumoconiosis produced the conglomerate "tuberculomas" or coalescent lesions.

Case 2. The patient (No. 32 886), a Spanish-American male, born in 1894, was employed for eleven and one-half months during 1942 and 1943 in the diatomite industry as a bag houseman and packer, respectively. A chest roentgenogram obtained elsewhere in 1944 was

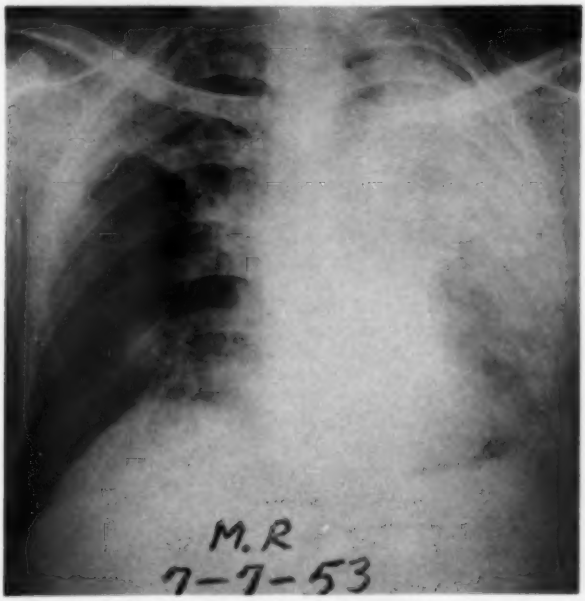

FIG. 3 (No. 32 886). A hospital admission film taken on July 7, 1953. This roentgenogram is identical in appearance with one taken on September 1, 1954, just preceding death. The lesion in the right upper lobe was interpreted by one observer as follows: "Coalescent lesion in right upper third now denser and further contracted toward the hilum."

FIG. 4 (No. 32 886). Coronal section of the right lung, showing contracted upper lobe with extensive pigmented coalescent scars, small pigmented scars in the middle lobe and the superior segment of the lower lobe, and lack of emphysema. Arrow marks solitary caseous lesion at medial surface of the lower lobe.

said to have been normal. An employment chest roentgenogram, obtained on February 26, 1946, revealed cavitation in the left second interspace. It was the opinion of a number of independent observers that definite bilateral pneumoconiosis of the linear type was present on this film. The patient was then admitted to the Santa Barbara General Hospital, where a diagnosis of active pulmonary tuberculosis was confirmed by the finding of tubercle bacilli in his sputum on microscopy and culture.

The patient's initial response to a left pneumothorax was excellent, his sputum became noninfectious, and he was discharged as an arrested case in 1947. The pneumothorax was later abandoned and he was readmitted to the hospital in December, 1950, because of hemoptysis and sputum positive for tubercle bacilli. Following a period of temporary improvement on antimicrobial therapy, the patient's course was slowly downhill with the subsequent development of cor pulmonale. He died on September 22, 1954.

A chest film obtained on July 7, 1953 (figure 3) is presented because it is identical in appearance with a chest film taken on September 1, 1954, just preceding death. Attention is directed to the "coalescent" lesion in the right upper lobe which was interpreted by one observer as follows: "Coalescent lesion in right upper third now denser and further contracted toward the hilum."

An autopsy was obtained and a portion of the pathologist's report is as follows:

> ". . . The right upper lobe is shrunken considerably and there is an irregular, hard, black, fibrous lesion that measures about 5 cm. in greatest diameter, which occupies the lateral and basal portions of the lobe." . . . "Representative microscopic sections of the right upper lobe show the following features: There are large stellate anastomosing areas of cellular fibrosis in the deeper tissue, and the intervening alveoli are emphysematous. The centers of some of these lesions are more or less hyalinized, and dust-laden cells infiltrate the interstitial spaces widely. There is a multinodular caseous lesion adjoining the pleura, which is encapsulated by hyaline fibrous tissue."

The pathologist commented, in part: "It was the opinion of all the consultants that

definite diffuse pneumoconiosis existed at the beginning of the tuberculous infection. This is supported by my findings in the lungs on pathological examination. The repeatedly positive sputum examinations and histological character of the coalescent pulmonary lesions confirm the role of tuberculous infection in their pathogenesis."

A photograph of a coronal section of the right lung (figure 4) shows the contracted right upper lobe and some of the pigmented, coalescent lesions.

Comment: A correlation of the clinical, roentgenographic, and pathologic findings in this case should leave little doubt but that tuberculous infection was a contributing factor in the coalescent disease in the right upper lobe. Conversely it is also evident that diatomaceous earth pneumoconiosis played a significant role in these lesions. It seems likely, therefore, that the coalescent lesions in this case are due to the combined action of tuberculosis and dust.

Case 3. The patient (No. 30 218), a white male, born in 1910, had worked in the diatomite industry from 1948 to 1953 as a car loader, a total dust exposure of four years and three months. A routine employment chest roentgenogram obtained on July 28, 1949, after six

FIG. 5 (No. 30 218). Employment chest roentgenogram obtained on February 11, 1953. This chest film was interpreted by three independent observers as follows:

(*1*) "P3 with marked coalescence in both apices.

(*2*) "Fibroid tuberculosis involving both apices.

(*3*) "Lighter technique (on film taken February 20, 1953) shows definite very tiny miliary densities occupying all parts of both lung fields. This is believed to have been present on the film of February 11, although much harder to see because of differences in technique."

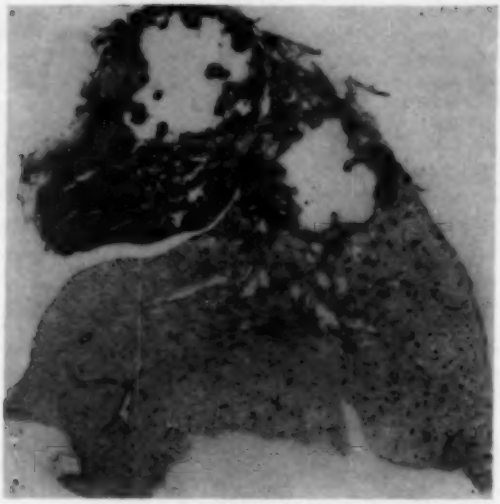

FIG. 6 (No. 30 218). Sagittal section of the left lung showing large cavities in the upper lobe and the apex of the lower lobe, contracted fibrous lingula containing caseous foci, and fibrocaseous nodules extending out from the lower lobe cavity. Note the lack of emphysema.

months' exposure to diatomaceous dust, was normal. Chest roentgenograms obtained on February 11, 1953 (figure 5) and February 20, 1953, were interpreted by two independent observers as follows: P3 with marked coalescence in both apices; fibroid tuberculosis involving both apices.

A reaction to first-strength PPD at this time was positive.

On May 31, 1953, the patient was admitted to the Santa Barbara General Hospital because of apparent pneumonia which failed to respond to antimicrobial therapy. A hospital admission chest film obtained on June 1, 1953, showed extensive acute exudative tuberculosis in the left lung. This diagnosis was confirmed by the finding of tubercle bacilli in the patient's sputum on both microscopy and culture. He was then placed on intensive antituberculous drug therapy consisting of dystricin (1.0 gm. twice weekly), PAS (4.0 gm. three times daily), and isoniazid (100 mg. every twelve hours). Because of the probability of a mixed infection in the contracted upper lobe, he was also given tetracycline initially in a dosage of 250 mg. every six hours. A chest roentgenogram obtained on October 29, 1954, after approximately eighteen months of continuous drug therapy, showed partial clearing of the disease in the left lung. A total left pneumonectomy was performed on November 5, 1954, by Dr. J. L. Robinson of Los Angeles. The patient was discharged from the hospital on May 7, 1955, with a diagnosis of inactive tuberculosis. He followed a drug regimen at home until the summer of 1956. When last seen in December, 1956, his disease was still classified as inactive.

The pathologist's report of the resected left lung is quoted in part as follows:

> "The lung is cut in sagittal planes. The apical and posterior portion of the upper lobe is almost entirely replaced by a large irregular cavity. . . . The surrounding pulmonary tissue in the posterior and apical portion of this lobe and the entire lingula are filled with caseous tubercles. . . . The histological findings may be summarized as follows. . . . Surrounding the cavities there are coalescent nodular caseous lesions. . . . In all these locations the caseous nodular lesions are separated by very cellular

tissue containing extremely scanty collagen. . . . The cells contain large amounts of fine granular brown pigment and numerous tiny colorless needle shaped and anisotropic dust particles."

A photograph of a sagittal section of the left lung was made (figure 6) showing the cavitary and caseous disease described above.

Comment: The clinical, roentgenographic, and pathologic findings in this case indicate that there can be no doubt of the presence of an active tuberculous infection and of the coexistence of pneumoconiosis due to diatomaceous earth dust. Attention is directed to the chest film obtained on February 11, 1953, preceding the development of the acute exudative tuberculosis (figure 5), which was interpreted differently by several observers. Since both entities, tuberculosis and pneumoconiosis, have been shown to coexist in the extensive left lung lesion which developed several months later, support is given to the premise that infection (tuberculous) may well have played a part in this early coalescent lesion.

Case 4. The patient (No. 22 630), a white male, born in 1910, worked from 1933 to 1939 as a car loader in the diatomaceous earth industry. For the next eight years he worked in the mill in the same industry, a total dust exposure of fourteen years. An employment chest roentgenogram obtained on August 18, 1939, was normal. Subsequent chest films taken in

FIG. 7 (No. 22 630). A hospital admission chest roentgenogram of 35-year-old white male, which was obtained on February 17, 1947. There is now a tuberculous cavity at the site of the coalescent lesion described previously. Sputum specimens were strongly positive for *M. tuberculosis*. The onset was acute two weeks preceding admission.

FIG. 8 (No. 22 630). Photograph of the right lung, cut in coronal plane, showing the huge cavity in the right upper lobe. Scattered, firm, encapsulated caseous lesions are present in the lower lobe. The microscopic findings confirmed the presence of widespread pneumoconiosis.

1942 and 1944 showed definite linear changes resembling progressive stages of diatomaceous earth pneumoconiosis. A routine employment roentgenogram obtained on April 17, 1946, revealed an infiltrative lesion in the right third interspace which was interpreted as beginning coalescence.

The patient had no complaints at this time. In 1945 he had been found to have a positive reaction to second-strength PPD. He continued to work until January, 1947, when he developed symptoms of an acute respiratory infection. His sputum was found to be positive for tubercle bacilli, following which he was hospitalized. A chest film taken on February 17, 1947 (figure 7) showed extensive disease in both lungs and a fluid-containing tuberculous cavity at the site of the previously described "coalescent lesion." In July, 1948, the patient was discharged from the hospital with clinically inactive tuberculosis. He was readmitted in 1951 with reactivated disease, following which his clinical course was slowly downhill. Subsequently he developed cor pulmonale in 1955, along with severe pulmonary insufficiency, and he died on April 30, 1956. An autopsy was performed. A photograph of the right lung cut in coronal plane was made (figure 8), showing the large trabeculated cavity in the posterior half of the right upper lobe and the firm caseous nodules in the right lower lobe. A portion of the pathologist's report is as follows:

> "Microscopically, multiple sections from the right and left lungs show extensive replacement and destruction of the lung parenchyma by a necrotizing fibrosing granulomatous process, characterized by large areas of necrosis, hyalinization and fibrosis marginated by dense hyalinized fibrous tissue and a peripheral infiltrate of lymphocytes and histiocytes in large numbers. The central portions of the fibrotic zones show structureless masses of hyalinized fibrous tissue without evidence of a whorled pattern....
>
> "No fungi or acid-fast bacilli are demonstrable in special stains.
>
> "Hilar lymph nodes microscopically show extensive replacement of the normal lymphoid architecture by dense hyalinized fibrous tissue similar to that noted in the pulmonary parenchyma. In some nodes there is a marginal rim of residual lymphoid tissue which is infiltrated by large numbers of histiocytes, many containing coarsely granular or fibular unidentified material."

Comment: In this case with a clinical history of fourteen years' exposure to

diatomaceous earth dust, the appearance of linear roentgenographic changes (which are characteristic of this type of pneumoconiosis) and the demonstration of gross and microscopic pathologic lesions in both lungs, which are characteristic of combined tuberculosis and diatomaceous earth pneumoconiosis, serve to partially document the presence of this type of pneumoconiosis in the patient. The coexistence of pulmonary tuberculosis has been proved. Interest is directed to the development of the tuberculous cavity with a fluid level in figure 7 in the same area in which an asymptomatic coalescent lesion had been demonstrated in a film taken on April 17, 1946. It can hardly be doubted that both tuberculosis and diatomite dust played a part in the pathogenesis of this lesion.

Case 5. The patient (No. 35 594), a white male, born in 1905, was employed in the diatomite industry continuously (with the exception of five months in 1934) from 1932 until 1956. Routine employment chest roentgenograms were essentially normal from 1935 until 1942, when early changes characteristic of diatomaceous earth pneumoconiosis were first noted. These lesions remained fairly stable until 1951, when early coalescent lesions were first described in the apices of both lungs.

Slight but progressive roentgenographic changes were noted in these lesions on routine annual films until 1953, when a marked increase in the extent and density of the coalescent lesions in both upper lobes was observed. This is demonstrated on a film taken on February 27, 1953 (figure 9).

The patient stated that he had no subjective symptoms until October, 1953, when he awakened one night with an attack of acute dyspnea, which subsided gradually over a forty-eight-hour period. He was able to continue working although he was conscious of dyspnea when he engaged in moderate exertion. A routine chest roentgenogram obtained on May 11, 1955 (figure 10) showed the progression of the coalescent lesions over a two-year period. In October, 1955, he developed an upper respiratory infection which caused him to cough; this increased his dyspnea. After the subsidence of this infection he was advised to enter a hospital for observation, which he did on January 15, 1956.

During this period of hospitalization it was learned that his tuberculin reaction had been positive in 1950. A chest roentgenogram obtained on January 17, 1956, revealed further extension of the coalescent lesions. Sputum and gastric washings were negative for acid-fast bacilli on microscopy and culture. A three-second vital capacity was 2,567 cubic centimeters. Planigrams centered over the right upper lobe coalescent lesion were equivocal for tuberculous cavitary or caseous disease. A cervical lymph node biopsy was negative for tuberculosis but positive for diatomaceous dust reaction.

Following his discharge the patient returned to work but shortly thereafter developed an acute upper respiratory infection, following which his sputum became positive for acid-fast bacilli. He was readmitted to the Santa Barbara General Hospital on May 26, 1956, and a chest roentgenogram obtained two days later revealed an extension of disease in the right lung field. Since admission the patient has responded well to multiple-drug therapy. A chest film taken on December 13, 1956, showed marked clearing of the exudative disease in the right lung and further contraction on the left. By January, 1957, he was ambulatory, on graduated exercise, with sputum negative for tubercle bacilli, and had gained 25 pounds in weight.

Case 6. The patient (No. 35 177), a white female, born in 1909, was employed as a sacker in the diatomite industry from 1944 to 1951. At the end of this period her chest roentgenograms were said to have shown "P3 pneumoconiosis with coalescence." A reliable pulmonary history during this period was not obtained.

In April, 1955, the patient was referred to a hospital for a chest survey. A chest film taken

FIG. 9 FIG. 10

FIG. 9 (No. 35 594). A routine employment chest roentgenogram of a white male born in 1905, obtained on February 27, 1953, showing the characteristic linear and coalescent changes which developed gradually over an approximate eleven-year period. The patient's tuberculin reaction was reported as positive in 1950. He did not complain of dyspnea or have any respiratory symptoms until October, 1953.

FIG. 10 (No. 35 594). Routine employment chest roentgenogram of the patient in figure 9, obtained on May 11, 1955. There has been considerable progression of the disease process. Note the extensive coalescence. The patient was still working as a foreman at this time and his only complaint was dyspnea on exertion. He could still climb a flight of stairs slowly without stopping.

FIG. 11 (No. 35 177). A hospital roentgenogram of a 45-year-old white female, obtained on April 14, 1955, while the patient was under observation. The coalescent shadows in both lungs are interesting in their resemblance to those in the preceding cases. This woman's only complaint was dyspnea on exertion. Her tuberculin reaction was positive.

on April 14, 1955 (figure 11) showed extensive "bilateral pneumoconiosis with emphysema." Presumably because of her occupational history, tuberculosis had not been considered previously as a possible diagnosis. The patient's chief and only complaint on admission, aside from mental depression, was shortness of breath on exertion. The degree of ventilatory pulmonary insufficiency is evidenced by the fact that her maximal breathing capacity was 41.0 liters per minute and her timed vital capacity was 1,921 cubic centimeters. Although her reaction to first-strength PPD was strongly positive, her sputum and gastric washings were consistently negative for acid-fast bacilli. Her general condition was excellent and she remained ambulatory throughout her hospital stay. She has never received antituberculous drugs.

The patient was discharged in October, 1955, but was readmitted on November 1, 1955, because she was unable to adjust psychologically to her home environment. She resumed her previous ambulatory status. This admission was uneventful except for the presence of transitory wheezing rales, and the patient was discharged on May 16, 1956. A chest roentgenogram obtained on September 15, 1955, was unchanged. Aside from dyspnea, this patient has had no pulmonary complaints whatsoever. When last heard from early in 1957 she was active and apparently well.

Comment: Cases 5 and *6* have been presented together because, although none of the roentgenographic changes in either case have been identified by pathologic studies, the similarity of the roentgenographic lesions in both cases to those described in the 4 previous cases justifies some preliminary conclusions. Active pulmonary tuberculosis has been proved to be present in *Case 5*, approximately five years after the patient was first found to have a positive tuberculin reaction, and the presence of diatomaceous dust was proved by a lymph node biopsy. On the basis of the presence of a strongly positive tuberculin reaction in *Case 6* and the coalescent lesions which appeared similar, it might be predicted that this patient will eventually develop active superimposed tuberculous infection with sputum positive for tubercle bacilli.

Finally, sufficient evidence has been presented to support the premise that tuberculous infection has been a contributing factor in the pathogenesis of the "coalescent" lesions in both cases.

Case 7. The patient (No. 30 099), a white male, born in 1912, had worked intermittently outside the mill in the diatomite industry between 1934 and 1936. Between July, 1941, and February, 1943, he was employed continuously in the industry and, with the exception of a period of less than three weeks, he worked in the mill as a baghouseman. Employment roentgenograms obtained in 1935, 1941, and 1942 were interpreted as normal. In 1943, while in apparent good health, the patient left the industry voluntarily to take a job elsewhere. In 1945 he reported to Los Angeles for an army induction examination. Shortly thereafter he was notified of his rejection because of "far advanced active pulmonary tuberculosis," following which he was admitted to the Santa Barbara General Hospital on April 23, 1945. On admission he denied cough, expectoration, dyspnea, blood streaking, chest pain, or any other significant local or constitutional symptoms. The physical examination was essentially normal. His reaction to first- and second-strength PPD was negative and also to Old Tuberculin in a dilution of 1:10 (10 mg.).

The admission chest film, taken on April 23, 1945, showed "fibroid infiltration extending down to the hilum bilaterally." On May 5, 1945, the patient was discharged from the hospital as nontuberculous.

Subsequently he was seen at regular intervals at the outpatient clinic. He did not complain and was working steadily as a gas station attendant. A routine chest film, taken on January 15, 1947, showed that both hilar shadows had become slightly elevated since 1945 and there was also slightly increased elevation of the medial portions of both diaphragms. The radiologist's comment was as follows: "There is believed to be inflammatory disease present, type undetermined."

The patient had gained about 15 pounds during this period, and his only complaint was that he seemed to tire more easily with strenuous work. In September, 1948, he stated that he had given up deer hunting because of shortness of breath. Routine chest roentgenograms in 1948, 1949 (figure 12), and 1950 were unchanged. He was hospitalized for two days in September, 1950, because of an attack of severe back and posterior chest pain. An electrocardiogram was normal at this time.

In June, 1953, because of an increase in his dyspnea, pulmonary function studies were performed which revealed a moderate reduction in 3-second vital capacity along with a marked reduction in the maximal breathing capacity. Alveolar nitrogen studies after breathing oxygen showed a moderate degree of impairment in air distribution in the lungs. The residual air capacity was within normal limits. In addition, there was a marked drop in arterial blood oxygen saturation with the one minute step-up exercise test. Surprisingly, therefore, the patient did not have a significant degree of pulmonary emphysema. The decrease in oxygen saturation with exercise was attributed to perfusion of areas of the lung which were not ventilated.

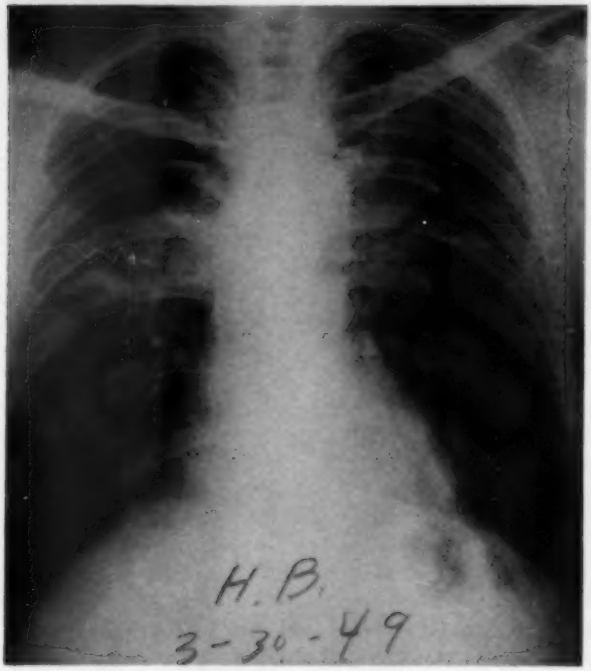

FIG. 12 (No. 30 099). A routine follow-up chest roentgenogram of a 37-year-old white male, obtained on March 30, 1949. The extensive hilar shadows had not changed appreciably since 1945, at which time they were interpreted at an army induction center as "far advanced pulmonary tuberculosis." The patient's tuberculin reaction has remained consistently negative and the disease is presumably uncomplicated diatomaceous earth pneumoconiosis, i.e., without superimposed infection. Note the difference in the location of these coalescent lesions as compared to those in the preceding case.

Comment: As of February 1, 1957, the patient is still alive and active. He has been advised to limit himself physically because of his dyspnea. He has not been helped appreciably by intermittent positive pressure breathing therapy. He receives a tuberculin test every six months and these have remained normal. His roentgenograms, obtained at regular intervals, have revealed surprisingly little change. A film taken on July 9, 1956, showed little change since 1948 except for some increase in density and contraction upward of the "coalescent" shadows adjacent to both hilar areas.

Case 8. The patient, a white male, born in 1906, was employed continuously in the diatomite industry from 1935 to 1941. With the exception of two periods totaling four months, he worked in the mill as a packer and leaderman. An employment chest roentgenogram obtained on August 2, 1935, was normal. Roentgenograms obtained in 1937, 1938, and 1939 were interpreted by one observer as showing a gradual increase in fibrosis in the upper half of both lung fields which was uniform and symmetrical. The patient stated that he first

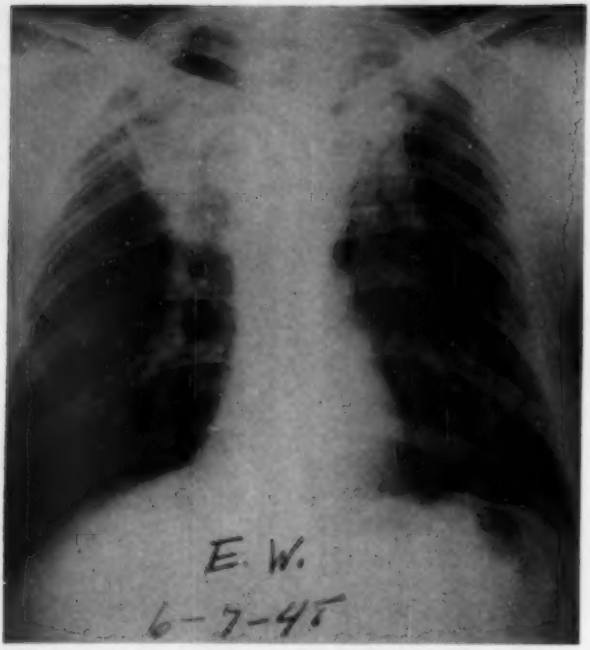

FIG. 13. Pre-employment hospital chest roentgenogram of a white male, 42 years of age, obtained on June 7, 1948. This patient had been exposed to diatomaceous earth dust from 1935 to 1941. Note the extensive symmetrical coalescent shadows which have been retracted upward along with both hilar structures. This patient has a consistently negative tuberculin reaction, and the pneumoconiosis in this case is considered to be uncomplicated by infection, tuberculous or otherwise.

began to notice excessive fatigue and shortness of breath in 1939. He continued to work, however, until 1941, when he left the industry of his own volition to work in Nevada. Later he returned to the area and consulted a physician because of an increase in his dyspnea. A chest film showed a marked increase in the previously described "fibrosis" which was still bilateral and symmetrical.

In February, 1944, he was hospitalized and was studied extensively by a lung specialist who submitted a report, the opening statement of which is as follows: "My diagnosis in this case is (*1*) far advanced, bilateral, pulmonary tuberculosis; (*2*) pneumoconiosis, mild, of the linear nonspecific type." Repeated gastric cultures were negative for tubercle bacilli.

In 1948 the patient applied for work as a hospital maintenance man. A pre-employment chest film, taken on June 7, 1948 (figure 13), revealed extensive symmetrical fibrotic and coalescent lesions in both upper lobes. Tuberculosis was immediately suspected. His only complaint, however, was dyspnea, and a reaction to PPD was negative in both dilutions. He worked at the maintenance job for a year or two, following which he left to accept an easier job as a school janitor. He reports irregularly for follow-up examinations. His most recent chest film, taken on May 29, 1956, revealed a definite increase in the density of the coalescent shadows since 1948. His tuberculin tests have remained consistently negative.

Although he is markedly disabled because of dyspnea and is prostrated by mild upper respiratory infections, he was still working as of February 1, 1957.

Comment: These 2 cases have been presented together because the patients are the only 2 with apparent uncomplicated or pure diatomaceous earth pneumoconiosis whom the present writer has examined and followed for a ten-year period. The similarity of their chest roentgenographic findings is immediately apparent. Particular attention is directed to the symmetrical, equilateral distribution of the coalescent roentgenographic densities in both cases. These are undoubtedly coalescent lesions of diatomaceous earth pneumoconiosis which have not been influenced by infection, tuberculous or otherwise. The dissimilarity of these coalescent lesions to those described in the first 6 cases is also immediately apparent.

Discussion

It is not within the scope of this paper to comment on any influence, favorable or otherwise, which the presence of diatomaceous earth pneumoconiosis may have on the clinical course of coexisting pulmonary tuberculosis. This is the subject of a separate study currently in progress. Fortunately, a number of such patients were observed sufficiently long in the pre-drug era to provide interesting material.

The present writer is fully aware that it is indeed presumptuous to assume that the roentgenographic appearance of the coalescent lesions in *Cases 7* and *8* is typical of all uncomplicated diatomaceous earth cases. On the other hand, it would seem advisable to separate these cases into groups with negative and positive tuberculin reactions to determine whether there is a pattern in the two groups. Such a study might provide prognostic data for radiologists.

Serious consideration should be given to the advisability of giving antituberculous chemotherapy to any patient with an occupational history and chest roentgenographic changes attributed to diatomaceous dust who has a positive tuberculin reaction.

It has been interesting to note the interpretation given to the various roentgenographic changes in these patients by radiologists viewing them for the first time. Universally they have listed tuberculosis as the first and probable diagnosis even in those patients with negative tuberculin reactions. Similarly the first-stage (P1) linear lesions described by those who have looked at these chest films long enough to have been indoctrinated are not described at all in this manner by visiting radiologists. More often they are considered to be normal films—a fact that should indeed give some pause for thought.

Summary

Clinical, roentgenographic, and pathologic data in 4 cases have been correlated to show that both tuberculosis and diatomaceous earth dust played a part in the pathogenesis of so-called coalescent lesions.

Two cases have been presented, one of a patient with proved active pulmon-

ary tuberculosis and one with a positive tuberculin reaction. Both patients had long exposure to diatomaceous earth dust and had roentgenographic coalescent changes similar in appearance to those of the first 4 cases. It is believed that sufficient data have been presented to justify a preliminary conclusion that both tuberculosis and diatomaceous earth dust have played a part in the development of the presumptive coalescent lesions of these 2 patients.

The only 2 patients with uncomplicated diatomaceous earth pneumoconiosis, i.e., negative tuberculin reactions, whom the present writer has followed clinically have coalescent roentgenographic changes which are strikingly similar in their symmetrical distribution. Their dissimilarity to the first 6 cases is apparent. Support is given to the premise that in uncomplicated pneumoconiosis of this type the lesions follow a different pathologic and roentgenographic pattern.

It can no longer be stated with assurance that infection has not contributed to the production of roentgenographic coalescent lesions in patients with this type of pneumoconiosis who have positive tuberculin reactions.

Hundreds of workers have been exposed to diatomaceous earth dust and many of them leave the area. Therefore, clinicians should be familiar with the clinical and roentgenographic characteristics of this most interesting entity.

Sumario

La Lesión Coalescente de la Neumoconiosis Debida a Tierra Diatomácea

Se han correlacionado los datos clínicos, radiográficos e histopatológicos de 4 casos para demostrar que tanto la tuberculosis cuanto la tierra diatomácea desempeñaron un papel en la patogenia de las llamadas lesiones coalescentes.

Se presentan 2 casos, uno con tuberculosis pulmonar activa comprobada y uno de reacción positiva a la tuberculina. En ambos enfermos había larga exposición a la tierra diatomáceay alteraciones coalescentes radiográficas de aspecto semejante a las de los primeros 4 casos. Parece que se han ofrecido datos suficientes para justificar la conclusión preliminar de que tanto la tuberculosis como la tierra diatomácea deben haber participado en la producción de las lesiones coalescentes presuntivas de estos 2 enfermos.

Los únicos 2 enfermos con neumoconiosis incomplicada debida a tierra diatomácea, es decir, con reacciones negativas a la tuberculina, que el A. haya seguido clínicamente, tienen lesiones radiográficas coalescentes que son notablemente semejantes en su distribución simétrica. Su desemejanza a los primeros 6 casos es manifiesta. Esto presta apoyo a la proposición de que, en la neumoconiosis incomplicada de esta forma, las lesiones se conforman a un distinto patrón histopatológico y roentgenográfico.

No cabe ya afirmar con seguridad que la infección no haya contribuído a la producción de lesiones coalescentes radiográficas en enfermos de esta forma de neumoconiosis que tienen reacciones positivas a la tuberculina.

Centenares de trabajadores se han visto expuestos a polvo de tierra diatomácea y muchos de ellos abandonan la zona, por lo cual los clínicos deben familiarizarse con las características clínicas y radiográficas de entidad tan interesante.

Resume

La lésion coalescente de la pneumoconiose due à la poussière de diatomées

Les données cliniques, radiologiques et anatomo-pathologiques de 4 cas ont été confrontées; elles démontrent que la tuberculose et la poussière de diatomées jouent toutes deux un rôle dans la pathogénèse de ce que l'on dénomme lésions coalescentes.

Deux cas sont présentés, l'un est une tuberculose pulmonaire évolutive évidente, l'autre montre une réaction positive à la tuberculine. Les deux malades ont été exposés pendant longtemps à la poussière de diatomées et leurs radiogrammes présentent des images de lésions coalescentes analogues à celles des quatre premiers cas. L'auteur considère que l'on dispose de données suffisantes pour justifier une conclusion préliminaire: la tuberculose et la poussière de diatomées ont l'une et l'autre participé au développement des lésions coalescentes chez les deux malades.

Les deux seuls malades atteints de pneumoconiose non compliquée due à la poussière de diatomées, c.a.d. avec réaction négative à la tuberculine, ont été suivis cliniquement par l'auteur. Chez ces malades, les aspects radiologiques des lésions coalescentes sont étonnamment semblables quant à leur répartition symétrique.

La conception de l'évolution de ces lésions selon des modalités pathologiques et radiologiques différentes dans cette forme de pneumoconiose non compliquée est appuyée par l'auteur.

Il n'est plus possible d'affirmer que l'infection tuberculeuse ne puisse contribuer à la production des lésions coalescentes décelées radiologiquement chez les malades présentant ce type de pneumoconiose et dont les réactions à la tuberculine sont positives.

Des centaines d'ouvriers sont exposés aux poussières de diatomées, et comme beaucoup d'entre eux quittent la région où le sol contient ces algues, les cliniciens doivent se familiariser avec les caractéristiques cliniques et radiologiques de cette intéressante entité.

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A RAPID MEANS OF DIFFERENTIATING ATYPICAL ACID-FAST BACILLI AND AVIAN TUBERCLE BACILLI FROM OTHER MYCOBACTERIA[1, 2, 3]

The Neotetrazolium Inhibition Test

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Tubercle bacilli have been grown only occasionally in the presence of neotetrazolium (1, 2). Antopol and coworkers (3) mention that this substance is toxic for tubercle bacilli at a concentration of 0.6:10,000 in Petragnani medium, while Mudd and associates (4) and Winterscheid and co-workers (5) use about the same concentration in American Trudeau Society medium to detect growth.

In preliminary studies, it was observed that various strains of mycobacteria reacted very differently in the presence of a given concentration of this compound. An atypical strain would grow abundantly and reduce neotetrazolium, while a human strain would be completely inhibited by the same concentration of this substance.

It was thought, therefore, that a study of the action of neotetrazolium on different types of mycobacteria growing in Tween®-albumin liquid medium might be useful as a means of differentiating strains.

Methods

Cultures employed (tables 1 and 2): A sterile solution of neotetrazolium was added to Tween-albumin medium (pH 6.8) in 16 by 125 millimeter screw-capped tubes. The volume in each tube was 5 ml. and the final concentration of neotetrazolium varied from 1:5,000 to 1:1,280,000 in doubling dilutions. Two tubes of each dilution and two control tubes of medium without neotetrazolium were inoculated with 2 drops of a fresh culture (0.05 optical density). The tubes were incubated at 37° C. and growth was measured in all tubes (Coleman Jr. spectrophotometer, 640 μ), after thorough agitation, when growth in the control tubes reached the standard 0.05 optical density.

The inhibitory concentration of neotetrazolium was chosen as that amount which produced an optical density inferior to the 0.05 standard optical density of the controls. The inhibitory concentration thus defined is higher than the minimal inhibitory concentration because the optical density measured in the tubes containing neotetrazolium is the sum of the optical density due to reduced neotetrazolium (purple color) and the optical density due to bacterial growth, while in the control tubes the optical density results only from bacterial growth.

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[2] Presented before the Medical Session, as part of *Section 2B*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 6, 1957.

[3] The present study is part of the cooperative study on atypical mycobacteria sponsored by the Laboratory Subcommittee of the American Trudeau Society, and has been aided by grants from the Wisconsin Anti-Tuberculosis Association, Milwaukee, Wisconsin.

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TABLE 1

INHIBITORY ACTION OF NEOTETRAZOLIUM FOR MYCOBACTERIA

	Inhibitory concentration of neotetrazolium
*Atypical strains**	
1 photochromogen, *Case 1*, Buhler and Pollak (7)	1:5,000
3 nonphotochromogen	1:5,000
4 photochromogen	1:5,000
6 scotochromogen	1:10,000
5 scotochromogen	1:10,000
8† photochromogen, *Case 2*, Wolinsky *et al.* (8)	1:10,000
7 nonphotochromogen	1:20,000
2 nonphotochromogen	1:20,000
Avian strain	
Virulent avian (isolated from fowl)	1:10,000
Mycobacterium fortuitum Cruz (9, 10)	1:80,000
Bovine strains	
Virulent bovine strain: source, U. S. Bureau of Animal Industry	1:80,000
BCG, Phipps strain	1:160,000
Saprophytic strains	
M. phlei	1:160,000
M. smegmatis, Ruth Gordon (11)	1:320,000
Human strains	
H37Rv	1:1,280,000
H37Ra	1:1,280,000
Recent sputum isolate	1:1,280,000

* Strains and classification obtained from the Laboratory Subcommittee of the American Trudeau Society. The classification is discussed by Timpe and Runyon (6).

† Approximate value, growth not homogeneous.

A strain of Nocardia was also tested, but it did not grow homogeneously. Judging roughly by comparison with the control, it may be concluded that 1:40,000 neotetrazolium concentration is inhibitory for this strain.

In addition, several preliminary experiments were performed with various dilutions of neotetrazolium incorporated in the solid medium of Middlebrook (7H-10). Duplicate plates of each dilution of neotetrazolium and a pair of control plates were inoculated with 0.1 ml. of a 10^{-3} dilution of a seven-day culture of the strain to be studied. The inoculum was then spread over the surface of the plate by means of a bent glass rod. The plates were incubated at 37° C.

RESULTS

From the data in table 1, it may be seen that neotetrazolium is about 100 times more inhibitory for human strains than for atypical and avian strains. The other types of mycobacteria are situated between these two extremes but can easily be differentiated from atypical and avian strains.

The neotetrazolium inhibition test has also been applied to the study of two other strains of *M. fortuitum* and to the study of a collection of other unknown cultures obtained from the Laboratory Subcommittee of the American Trudeau Society. The only fact that was known about this series of cultures at the time

TABLE 2

INHIBITORY ACTION OF NEOTETRAZOLIUM FOR MYCOBACTERIA

I. *Atypical and avian strains*
  A. *Strains for which a 1:10,000 neotetrazolium concentration is not inhibitory*
    16 photochromogen, *Case 2*, Buhler and Pollak (7)
    26 photochromogen
    24 photochromogen
    22 photochromogen
    21 photochromogen
    19* scotochromogen
    20 nonphotochromogen
    14 *M. avium* (Kirschberg)
  B. *Strains for which a 1:40,000 neotetrazolium concentration is not inhibitory*
    18 photochromogen
    23† photochromogen and nonphotochromogen
    25 nonphotochromogen
II *Various strains for which a 1:40,000 neotetrazolium concentration is inhibitory*
  15 scotochromogen
  17 nonphotochromogen
  11 *M. phlei*
  9 H37Rv
  10 H37Ra
  12 R1Rv
  13 Bovine Ravenel isoniazid-resistant
    M. fortuitum 12 (Kushner)
    M. fortuitum 389 (Gordon)

* Growth not homogeneous.
† After performance of the test, it was learned that strain 23 is a mixed culture.

these experiments were performed was that the collection contained different types of atypical strains as well as examples of known varieties of mycobacteria. Neotetrazolium was used in concentrations of 1:10,000 and 1:40,000 and the usual controls without neotetrazolium were included. The results obtained with this series of cultures are shown in table 2.

With these cultures also the difference between the atypical-avian group and other mycobacteria is obvious. Among the known human, bovine, saprophytic, and *M. fortuitum* strains, no strain has been found which was not inhibited by 1:40,000 concentrations of neotetrazolium. Among the avian and atypical strains, only strains 15 and 17 were inhibited by this same concentration of neotetrazolium. Further experiments will be necessary in order to establish the position of strains 15 and 17 in the classification of mycobacteria.

The results in tables 1 and 2 also show that the photochromogenic atypical strains are not inhibited by 1:10,000 concentrations of neotetrazolium except for strain 18 which is only slightly inhibited. The scotochromogenic and the nonphotochromogenic strains vary in their susceptibility to this same concentration of neotetrazolium but they are generally more susceptible than the photochromogens.

Preliminary results on the solid medium of Middlebrook have confirmed the difference observed between human and atypical strains. Examples of the type

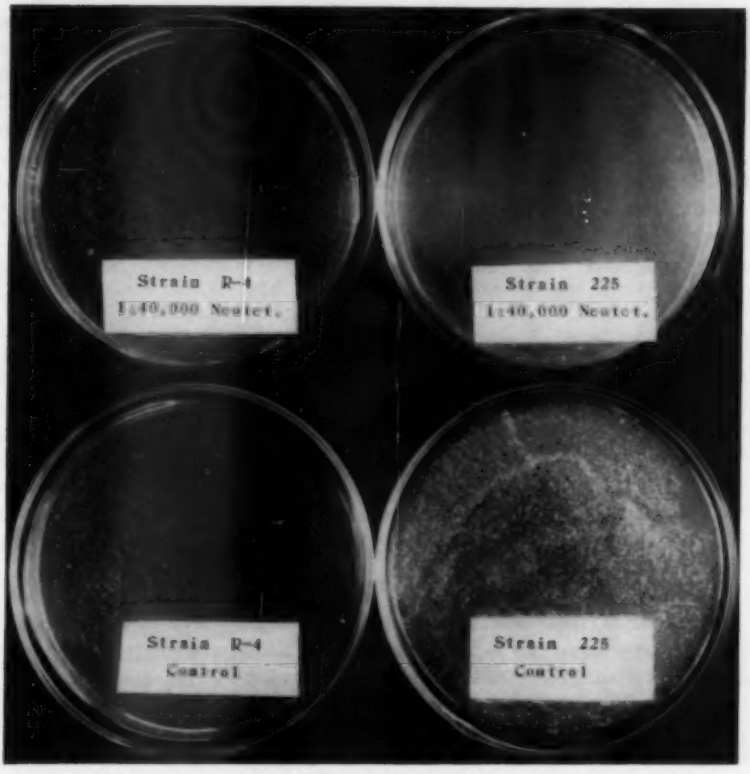

FIG. 1. Differentiation of a human strain from an atypical strain by means of neotetrazolium.

of inhibition seen with one photochromogenic atypical strain (strain R*4*) and one human strain (strain *225*) are shown in figure 1. After eighteen days' incubation, marked reduction may be seen in the growth of the human strain, while the atypical strain appears to be unaffected.

DISCUSSION

Several *in vitro* tests have been suggested as aids in the classification of mycobacteria (12–33). Some of these, like the analysis of lipid content (12), the bacteriophage typing (13, 14), the study of growth patterns in HeLa cells (15, 16), or the microcolonial test (17, 18), permit the differentiation of the usual mammalian bacilli from atypical strains. Nevertheless, the neotetrazolium inhibition test is useful because of its simplicity and rapidity.

In studies concerning the characterization of atypical tubercle bacilli by their lipid content, strains *1*, *4*, *16*, and *21* were classified as photochromogenic atypical strains, strain *20* as probably avian, and strains *15* and *19* as probably saprophytic. These conclusions are not contradicted by the results of the neotetrazo-

lium test except for strain *19*. As strain *19* does not grow very homogeneously in Tween®-albumin medium, the results of the neotetrazolium test on this strain are not very accurate and will have to be confirmed by other tests before any conclusions can be drawn.

Froman's experiments concerning the bacteriophage typing of atypical and other acid-fast organisms are also confirmed except for the data on strains *15* and *17*. He finds these strains to be resistant to the phages and considers them to be atypical strains of the avian type. It is possible that these strains are limit cases which might make the transition between the atypical-avian group and other mycobacteria.

Summary

The neotetrazolium inhibition test was performed on a total of 20 strains of mycobacteria thought to be atypical and on 15 other known strains of mycobacteria, including human, bovine, and avian varieties. Both virulent and attenuated strains were included. On the basis of their inhibition by neotetrazolium, these strains were divided into two groups: (*1*) human, bovine, saprophytic, and *M. fortuitum* strains which did not grow in the presence of neotetrazolium in a concentration greater than 1:40,000; and (*2*) atypical and avian strains which were capable of growing in concentrations of neotetrazolium greater than 1:40,000.

This simple test can be applied most usefully in laboratory diagnosis in which the presence of atypical or avian strains cannot usually be assessed before time-consuming experiments have been completed. The differentiation of atypical or avian strains from human strains is particularly easy by this method.

Sumario

Medio Rápido de Diferenciar los Bacilos Acidorresistentes Atípicos y los Bacilos Tuberculosos Aviarios de Otras Micobacterias: La Prueba de la Inhibición por Neotetrazolio

La prueba de la inhibición con neotetrazolio fué ejecutada en un total de 20 cepas consideradas atípicas y en otras 15 cepas conocidas de micobacterias comprendiendo variedades humanas, bovinas y aviarias. Entre ellas había cepas virulentas y atenuadas. A base de su inhibición por el neotetrazolio, se dividieron estas cepas en dos grupos: (*1*) cepas humanas, bovinas, saprofitas y *M. fortuitum* que no proliferan cuando el neotetrazolio alcanza una concentración superior a 1:40,000 y (*2*) cepas atípicas y aviarias que son capaces de formar colonias a concentraciones de neotetrazolio que pasan de 1:40,000.

Esta sencilla prueba alcanza su mayor utilidad en el diagnóstico de laboratorio cuando no se puede por lo general determinar la presencia de cepas atípicas o aviarias sin experimentos prolongados. La diferenciación de las cepas atípicas o aviarias de las humanas es en particular fácil con este método.

Resume

Moyen rapide de différencier les bacilles acido-résistants atypiques et les bacilles tuberculeux aviaires des autres mycobactéries: le test d'inhibition au néotétrazole

Le test d'inhibition au néotétrazole a été effectué sur un total de 20 souches considérées comme étant atypiques et sur 15 autres souches connues de mycobactéries, y compris les variétés humaines, bovines et aviaires ainsi que des souches virulentes et des souches atténuées. En considérant le pouvoir inhibiteur du néotétrazole à l'égard de ces souches,

ces dernières ont été divisées en 2 groupes: (1) Souches humaines, bovines, saprophytes et *M. fortuitum* qui ne se développent pas en présence de néotétrazole à 1/40.000; (2) souches atypiques et aviaires qui peuvent croître en présence de concentrations de néotétrazole plus élevées que 1/40.000.

Ce test simple peut être appliqué utilement dans le domaine du diagnostic de laboratoire où habituellement la présence de souches atypiques ou aviaires ne peut être affirmée avant que des expériences de longue durée aient été éxécutées. La différenciation entre les souches aviaires ou atypiques et les souches humaines est particulièrement aisée grâce à cette méthode.

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DIFFERENTIATION OF HUMAN TUBERCLE BACILLI FROM ATYPICAL ACID-FAST BACILLI[1,2]

I. Niacin Production of Human Tubercle Bacilli and Atypical Acid-fast Bacilli

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Introduction

The probem of pulmonary infection with atypical acid-fast bacilli has received increasing attention (1). The majority of reports are concerned with methods to distinguish the atypical acid-fast bacilli from human tubercle bacilli.

Konno (2) and Konno, Kurzman, and Bird (3), using the cyanogen bromide method, measured the niacin production of human, bovine, and avian tubercle bacilli and nonpathogenic acid-fast bacilli in the heat-extracted and hydrolyzed culture filtrates of synthetic liquid medium. Human tubercle bacilli produce markedly more niacin than the others. Niacin production is unrelated to virulence. Konno (4) has devised a simple modification of this method, which is applicable to bacilli obtained directly from solid medium. In both of these procedures, the indirect (3) and the direct (4), the niacin is determined by chemical methods. Another accepted method of determining niacin production is the microbiologic assay technique which measures niacin utilization by *Lactobacillus arabinosus* (5).

It is the purpose of this paper to determine the difference in niacin production between human tubercle bacilli and atypical acid-fast bacilli, using the bioassay method and the chemical methods (indirect and direct) and comparing their merits, accuracy, and usefulness.

Materials and Methods

Materials for the comparison of the bioassay method with the chemical methods were: three stock laboratory strains of human tubercle bacilli and four strains of atypical acid-fast bacilli.

Human tubercle bacilli:

H37Rv, H37Ra, R1Rv } from Trudeau Laboratory, Trudeau, New York

Atypical acid-fast bacilli:

M.B. (from Trudeau Laboratory, Trudeau, New York)
D.H. (from Veterans Administration Hospital, Minneapolis, Minnesota)
F.P. (from Niagara Sanatorium, Niagara, New York)

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[1] From the Cambridge Sanatorium, Cambridge, Massachusetts; the Research Institute for Tuberculosis and Leprosy, Tohoku University, Sendai, Japan; and the Department of Bacteriology and Immunology, Harvard University Medical School, Boston, Massachusetts.

[2] This paper was presented, in part, before the Medical Session, as part of *Section 2B*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 6, 1957.

These three strains were photochromogens which were repeatedly found in culture from sputum and from resected lung specimens.

A.W. (from Battey State Hospital, Rome, Georgia)

This strain is nonchromogenic and was cultured from the resected lung specimen.

Synthetic liquid culture medium: Fifty milliliters of a modified Sauton synthetic liquid medium[3] were used (3). All strains were inoculated on the surface for thirty days. After the incubation period the whole culture was heat-extracted at 100°C. for one hour. This extracts the niacin from the body of the bacilli into the culture medium. The extract was then filtered through filter paper. The volume was readjusted to 50 ml.

Solid medium: Löwenstein-Jensen medium was used. All strains were incubated for two months.

Methods used: The determination of niacin by bioassay using *Lactobacillus arabinosus* 17-5[4]: The method of analysis of the Association of Official Agricultural Chemists (5) was used. For the performance of the bioassay method, 0.1 ml. of a diluted culture filtrate was employed. Because of the higher niacin content of human tubercle bacilli, a 100 to 200 times dilution was prepared; whereas in the case of atypical acid-fast bacilli, a 20 to 50 times dilution was found to suffice. For the bioassay of bacilli taken directly from solid medium the bacillary inoculum was weighed and then emulsified with 10 ml. of physiologic saline solution. For the test, 0.01 ml. of the emulsion of human tubercle bacilli and 0.1 ml. of the emulsion of the atypical acid-fast bacilli were used.

The determination of niacin content by the cyanogen bromide method, using a liquid culture ("indirect" chemical method), was previously described by the writers (3). The culture filtrate was hydrolyzed with sodium hydroxide. This procedure converts the niacin precursor to niacin. One milliliter of hydrolyzed culture filtrate was used for the determination of niacin with cyanogen bromide and ammonia buffer.

Chemical determination of niacin from bacilli taken from solid medium ("direct" chemical method): Two to three loopfuls of bacilli are taken from the solid culture medium, weighed, and then placed into 1 ml. of 4 per cent aniline solution in 95 per cent alcohol. One milliliter of 10 per cent cyanogen bromide in water is added. The bacterial suspension is filtered through filter paper. A yellow color indicates niacin. This color is immediately measured electrophotometrically. (In this study quantitative determination was done for comparison with the bioassay technique. Weighing the bacilli and the electrophotometric determination may be omitted for the qualitative test. A developing yellow color is then directly compared with blank control.)

As a blank control, bacilli in 1 ml. of aniline solution and 1 ml. of water are used.

Results

There was a marked difference in the amount of niacin produced by human tubercle bacilli compared with the niacin production of atypical acid-fast bacilli (table 1). The lowest value in niacin production of human tubercle bacilli was 11.8 γ per 1 mg. of dry bacilli. The highest value in atypical acid-fast bacilli was 1.1 γ per 1 mg. of dry bacilli. The difference in niacin production of human tubercle bacilli and atypical acid-fast bacilli is more clearly demonstrated in figure 1.

A close parallel was found for the results obtained with the two different methods, the bioassay and the indirect chemical method. The results obtained

[3] The original Sauton formula contains ferric ammonium citrate 0.06 gm. This imparts a slightly yellow color to the medium and was therefore omitted to prevent misinterpretation of the yellow color developing during a positive test. This omission does not alter the growth of mycobacteria.

[4] Obtained from American Type Culture Collection, 2029 M St. N.W., Washington, D. C. as No. 8014.

TABLE 1

NIACIN CONTENT OF 50 ML. OF CULTURE FILTRATE FROM MODIFIED SAUTON LIQUID MEDIUM

Bioassay (*L. arabinosus*) vs. Chemical Method (Cyanogen Bromide)

	Strains	Dried Bacilli	Bioassay		BrCN	
			Niacin γ/ml. Filtrate	Niacin γ/mg. Dry Bacilli	Niacin γ/ml. Filtrate	Niacin γ/mg. Dry Bacilli
		mg.				
Human	H37Rv	138	48.5	17.6	44.0	15.8
	H37Ra	116	39.0	16.8	32.0	13.8
	R1Rv	150	39.0	13.2	35.0	11.8
Atypical	M.B.	128	2.7	1.1	3.0	1.1
	A.W.	104	1.0	0.5	1.0	0.5
	F.P.	105	2.5	1.0	1.0	0.4
	D.H.	126	2.0	1.0	1.0	0.5

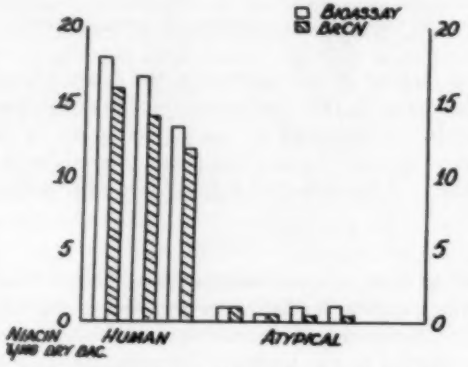

FIG. 1. Niacin content of the culture filtrate from modified Sauton liquid medium. Bioassay (*L. arabinosus*) vs. cyanogen bromide (BrCN).

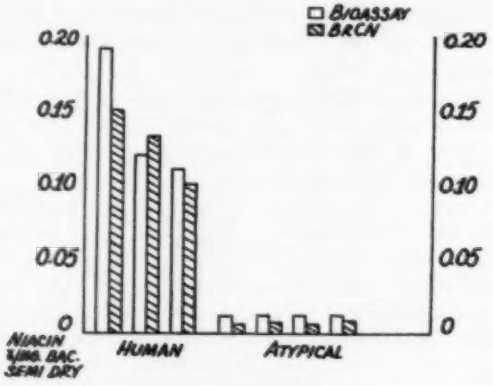

FIG. 2. Niacin content of the bacilli on routine solid culture medium. Bioassay (*L. arabinosus*) vs. cyanogen bromide (BrCN).

TABLE 2

NIACIN CONTENT OF THE BACILLI ON ROUTINE SOLID MEDIUM
Bioassay (*L. arabinosus*) vs. Chemical Method (Cyanogen Bromide)

	Strains	Bioassay			Cyanogen Bromide		
		Semi-dry Bacilli*	Niacin	Niacin	Semi-dry Bacilli	Niacin	Niacin
		mg.	γ	γ/*mg. bacilli*	*mg.*	γ	γ/*mg. bacilli*
Human	H37Rv	50	9.50	0.19	56	8.5	0.150
	H37Ra	56	7.80	0.12	60	8.0	0.130
	R1Rv	58	6.30	0.11	71	7.0	0.100
Atypical	M.B.	53	0.50	0.01	58	0.3	0.005
	A.W.	69	0.60	0.01	51	0.3	0.006
	F.P.	41	0.50	0.01	57	0.3	0.005
	D.H.	48	0.75	0.01	68	0.5	0.007

* The bacilli grown on solid media are considered "semi-dry bacilli."

when the bioassay method is compared with the direct chemical method are shown in table 2 and figure 2. The close parallel of the two methods may be seen in the marked quantitative difference in niacin production by human bacilli as compared to atypical acid-fast bacilli. The niacin production of human tubercle bacilli was more then ten times that of atypical acid-fast bacilli.

DISCUSSION

Coenzymes such as di- or tri-phosphopyridine-nucleotide (DPN or TPN) are essential in carrying on oxidation-reduction processes in the cell. The functional part of DPN or TPN is niacin. Some bacteria are unable to synthesize niacin and, thus, it must be supplied to the medium. For example, *L. arabinosus* will not grow unless niacin is supplied. Other bacteria have the ability to synthesize niacin from simple amino acids which can be supplied to the medium and can grow without added niacin. These include mycobacteria. This ability and the difference in the niacin content among the mycobacteria were the subject of a previous paper (3).

This report delineates the difference in the niacin content of human tubercle bacilli and atypical acid-fast bacilli. The results further define the relative accuracy and usefulness of different techniques to measure the production of niacin and its derivatives by these bacteria.

There are two ways to measure niacin content, the microbiologic and the chemical methods. The microbiologic method is a bioassay technique measuring growth response of *L. arabinosus* to niacin. The growth of this bacillus is directly proportional to the niacin concentration in the medium. This method is highly accurate but is cumbersome and time-consuming for practical use. It was used in the present study to serve as a standard for the accuracy of the more practical chemical methods.

The chemical methods proposed are essentially based on Koenig's reaction,

i.e., the development of a yellow color when cyanogen bromide is added to pyridine derivatives (such as niacin) in the presence of amine. Niacin precursors can be converted into niacin by hydrolyzation.

Two modifications of a chemical test have been devised (3, 4), namely, (*1*) the quantitative "indirect" method which allows determination of the niacin content in the culture filtrate of modified Sauton liquid medium, and (*2*) the qualitative "direct" method of adding cyanogen bromide directly to the culture material obtained on solid routine medium.

Both methods have their merits and limitations. The indirect method is more accurate but requires time. The direct method is attractive as a short and practical way to estimate niacin. The resulting color, however, is not as strong as in the culture filtrate method. This is understandable if it is realized that the indirect method determines the total niacin content of a heat extract from the myocobacteria, whereas the direct method probably measures only the niacin present on the surface of the bacilli. This makes it necessary to use large samples of culture material in order to obtain clear-cut results. A yellow color develops only if at least 1 γ of niacin is present in the material tested. There is some spread in the range of niacin content of human tubercle bacilli but, according to table 2, it is evident that these amounts of niacin require at least 10 mg. of semidry bacilli. It was also found useful to read the test in reflected and not in transmitted light and to use fluorescent light as a light source.

The close parallel in the values obtained by the indirect and the direct methods, respectively, are clearly demonstrated in tables 1 and 2. The comparison of the chemical methods with the bioassay technique establishes them as sufficiently accurate for this study. The tables also demonstrate in clear fashion the marked and significant difference in the niacin levels of human and atypical acid-fast bacilli. These methods are thus established as a diagnostic tool for identifying acid-fast bacilli as human tubercle bacilli.

Summary

Human tubercle bacilli can be identified by a significantly increased niacin content. This can be measured in three ways:

A. Bioassay, using niacin utilization of *Lactobacillus arabinosus*. This is the most accurate method but is complicated in its performance. It was used in the present study for comparison with other methods.

B. Quantitative chemical determination of the niacin content in the culture filtrate of synthetic liquid medium (indirect test). This method compares very well with the bioassay method and is sufficiently accurate for most purposes.

C. Qualitative modification of the above chemical test using the bacilli directly taken from routine solid culture medium (direct test). This may be regarded as a quick test for clinical use or for screening purposes.

All three methods clearly demonstrate the marked difference in the niacin content of human tubercle bacilli and atypical acid-fast bacilli. They can, therefore, be used as reliable differential diagnostic tools.

Sumario

Diferenciación de los Bacilos Tuberculosos Humanos de los Bacilos Acidorresistentes Atípicos. Parte I. Producción de Niacina por los Bacilos Tuberculosos Humanos y los Acidorresistentes Atípicos

Los bacilos tuberculosos humanos pueden identificarse por un aumento significativo en su contenido de niacina. Este puede medirse de tres modos:

A. Biovaloración, usando la asimilación de niacina por el *Lactobacillus arabinosus*. Este es el método más exacto, pero su ejecución es complicada. Se introduce en este estudio para comparación con otros métodos.

B. Determinación química cuantitativa del contenido de niacina en el filtrado cultural de medios líquidos sintéticos (prueba indirecta). Este método se compara muy bien con el de biovaloración y posee una exactitud suficiente para la mayor parte de los fines.

C. Modificación cualitativa de la precedente prueba química usando los bacilos tomados directamente del cultivo sólido corriente (prueba directa). Esta puede considerarse como prueba rápida para empleo clínico o fines de triaje.

Los tres métodos demuestran claramente la notable diferencia en el contenido de niacina de los bacilos tuberculosos humanos y los bacilos ácidorresistentes atípicos, de modo que pueden usarse como instrumentos fidedignos para el diagnóstico diferencial.

Resume

Différenciation du bacille tuberculeux humain des bacilles acido-résistants atypiques. I. Production de niacine par les bacilles tuberculeux humains et bacilles acido-résistants atypiques

Le bacille tuberculeux humain peut être identifié grâce à la quantité notable de niacine qu'il contient. La détermination peut se faire de trois façons:

A. La détermination biologique, basée sur l'utilisation de la niacine par le *Lactobacillus arabinosus*: méthode plus précise mais d'exécution compliquée. Elle est introduite dans cette étude à titre de comparaison.

B. La détermination chimique quantitative de la teneur en niacine du filtrat du milieu synthétique liquide de culture (test indirect). Cette méthode soutient la comparaison avec la méthode de détermination biologique et est suffisamment précise pour convenir à la plupart des cas.

C. Une modification qualitative du test chimique ci-dessus, où les bacilles sont directement prélevés dans un milieu de culture solide ordinaire (test direct). Ceci peut être considéré comme un test rapide à utiliser en clinique ou comme moyen de dépistage.

Ces trois méthodes démontrent nettement la différence entre la teneur en niacine du bacille tuberculeux humain et celle des bacilles acido-résistants atypiques. En conséquence, elles constituent un instrument sûr pouvant être utilisé dans le diagnostic différentiel.

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DIFFERENTIATION OF HUMAN TUBERCLE BACILLI FROM ATYPICAL ACID-FAST BACILLI[1,2]

II. Clinical Application

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Introduction

It has been shown in the preceding paper (1) that human tubercle bacilli produce remarkably more niacin than atypical acid-fast bacilli. This difference is significant enough to serve as a test to distinguish these bacilli.

The present paper concerns itself with the application to two studies of the chemical methods previously described: One study is the identification of 26 unknown strains of mycobacteria distributed by the Laboratory Subcommittee of the American Trudeau Society; the other is the investigation of 663 routine cultures from nine tuberculosis hospitals in the New England area. These cultures had been reported as positive for tubercle bacilli in the respective hospitals.

Materials and Methods

Materials for the clinical applications were:

Twenty six unknown strains of mycobacteria: The Laboratory Subcommittee of the American Trudeau Society on Atypical Acid-fast Bacilli (Dr. E. Wolinsky, *Chairman*) distributed 26 different strains of myocbacteria through the laboratory of the Sunmount Veterans Administration Hospital (Dr. E. Runyon) to a number of investigators. The bacilli were labeled by number only and were unknown to the investigators.

Six hundred and sixty-three routine cultures from nine tuberculosis hospitals in New England: These cultures were obtained from both treated and untreated patients and had been classified as positive for tubercle bacilli by the laboratories of the respective hospitals. The cultures were from two to six months old, and had been grown on various media (Löwenstein-Jensen, Petragnani, and American Trudeau Society).

Methods: Subculture on synthetic liquid medium and quantitative determination (indirect chemical method), as mentioned in a previous paper (2) was used.

In the qualitative "direct chemical method" (3), two to three generous loopfuls of bacilli were taken from solid medium but were not weighed. This inoculum was placed into 1 ml. of 4 per cent aniline solution in 95 per cent alcohol, and 1 ml. of 10 per cent cyanogen bromide in water was added. The developing yellow color was then directly compared with the blank control. No quantitative determination was performed. The buff pigment of the tubercle bacilli does not dissolve in the reagent. However, the orange pigment of some chromogens does dissolve in the reagent. This possible source of error can be eliminated easily by using a blank control.

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[1] From the Cambridge Sanatorium, Cambridge, Massachusetts; the Research Institute for Tuberculosis and Leprosy, Tohoku University, Sendai, Japan; and the Department of Bacteriology and Immunology, Harvard University Medical School, Boston, Massachusetts.

[2] This paper was presented, in part, before the Medical Session, as part of *Section 2B*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 6, 1957.

The identification of the "26 unknown strains of mycobacteria" was done by the direct and the indirect methods.

When using the indirect method in identifying the 26 unknown strains, some of the atypical acid-fast bacilli could not be cultured on the surface of the synthetic liquid medium because certain strains have a tendency to sink to the bottom of the culture fluid. Among the 26 strains, Nos. *2*, *4*, *8*, *9*, *10*, *11*, *12*, *13*, *14*, and *24* grew on the surface. The other strains grew abundantly at the bottom of the medium. Both sets of strains were filtered, and the culture filtrates were studied for niacin content.

For the identification of 663 routine cultures from nine tuberculosis hospitals, only the direct chemical test was performed.

If the cultures were scanty, subculture on routine solid medium was done in order to obtain enough material to perform the test. Among the 663 cultures examined, 7 atypical mycobacteria were found. In these cases guinea pig and mice inoculations followed. For the guinea pig inoculation, 0.2 ml. of a 14-day culture in Dubos albumin liquid medium was injected subcutaneously. For the mouse inoculation, 0.5 ml. of a 14-day culture in Dubos albumin liquid medium was injected intravenously. After six weeks all animals were sacrificed, including animals infected with H37Rv, which served as controls.

Results

The results of the examination of the 26 unknown strains from the Sunmount Cooperative Study may be seen in table 1.[3] Only the cultures of Nos. *9*, *10*, and *12* gave a positive direct test (yellow color). Hence these three were identified as *Mycobacterium tuberculosis* var. *hominis*. Repeated tests of the other 23 strains gave negative results.

To confirm this, the quantitative indirect chemical method was also done on both groups of bacteria. The strains labeled Nos. *9*, *10*, and *12* showed a niacin production of 26 γ, 22 γ, and 15 γ, respectively, per ml. of culture filtrate, whereas the highest value in the group of the remaining 23 strains was only 2.5 γ per ml.

Identification of Nos. *9*, *10*, and *12* as *M. tuberculosis* var. *hominis* was confirmed by the Sunmount Cooperative Group which specified them as H37Rv, H37Ra, and R1Rv.

The remaining 23 strains were atypical acid-fast bacilli with the exception of one strain each of *M. phlei*, *M. tuberculosis* var. *bovis* (Ravenel), an isoniazid-resistant strain, and *Mycobacterium tuberculosis* var. *avium* (Kirchberg).

The results of the direct chemical niacin test on 663 routine cultures from nine tuberculosis hospitals are tabulated in table 2.

Three hundred and sixty-two cultures (54.7 per cent) were identified as human tubercle bacilli by the direct test without subculture. Two hundred and ninety-four (44.3 per cent) required subcultures because of insufficient growth on the original culture. Two hundred and nine of these subcultures were identified as human tubercle bacilli. The remaining 85 strains did not grow sufficiently to perform the test or did not grow at all on subculture.

Therefore, 86.2 per cent of the 663 routine cultures examined gave a positive niacin test. Seven of these same 663 strains gave a negative niacin test. The

[3] As a control, 10 strains of nocardia obtained from Mr. James Gray, State Diagnostic Laboratory, Jamaica Plain, Massachusetts, were examined and gave a negative niacin test. Among these 10 strains, 3 were pathogenic.

TABLE 1

THE NIACIN PRODUCTION OF "26 UNKNOWN STRAINS" FROM THE SUBCOMMITTEE ON ATYPICAL ACID-FAST BACILLI

Strain Number	Niacin Test		Identified as:	Later Confirmed by "Cooperative Group" as:
	Direct Test (Qualitative)	Indirect Test (Quantatitive) Niacin γ/Ml. Culture Filtrate		
1	−	0.6	Non-human	Atypical
2	−	0.8	Non-human	Atypical
3	−	0.5	Non-human	Atypical
4	−	1.0	Non-human	Atypical
5	−	1.0	Non-human	Atypical
6	−	2.5	Non-human	Atypical
7	−	1.0	Non-human	Atypical
8	−	1.0	Non-human	Atypical
9	+	32.0	Human	Human H37Rv
10	+	22.0	Human	Human H37Ra
11	−	2.0	Non-human	*M. phlei*
12	+	15.0	Human	Human R1Rv
13	−	0.6	Non-human	Bovine, Ravenel, isoniazid-resistant
14	−	2.0	Non-human	Avian, Kirchberg
15	−	1.0	Non-human	Atypical
16	−	1.0	Non-human	Atypical
17	−	1.0	Non-human	Atypical
18	−	1.0	Non-human	Atypical
19	−	1.0	Non-human	Atypical
20	−	1.0	Non-human	Atypical
21	−	1.0	Non-human	Atypical
22	−	1.0	Non-human	Atypical
23	−	1.0	Non-human	Atypical
24	−	2.5	Non-human	Atypical
25	−	2.0	Non-human	Atypical
26	−	2.0	Non-human	Atypical

TABLE 2

ANALYSIS OF 663 PATIENT STRAINS REPORTED AS POSITIVE FOR TUBERCLE BACILLI (From Nine Tuberculosis Hospitals)

Total	Positive on Direct Test without Subculture	Insufficient Material Subcultured		Atypical Acid-fast Bacilli, Negative Test
		294 (44.3%)		
		Positive Test on Subculture	Uncertain Results (Unsatisfactory Cultures)	
	362 (54.7%)	209 (31.5%)		
663 (100%)	571 (86.2%)		85 (12.8%)	7 (1.0%)

original cultures of these 7 niacin-negative strains were difficult to distinguish from human tubercle bacilli. The chromogenic nature of these strains was easily demonstrated by subculture, and all strains were acid fast. One of the 7 strains grew on Sabouraud medium at room temperature and showed filamentous branching. This strain was therefore identified as nocardia. The other 6 strains were classified as atypical acid-fast bacilli.

Guinea pig inoculation with these 7 strains did not produce any lesions. A control guinea pig injected with the human strain of H37Rv showed generalized progressive tuberculosis with tubercles and caseation in the lungs, liver, and spleen. Four of these 6 strains of atypical acid-fast bacilli were injected into mice. No pathologic changes were noted. One of the remaining 2 strains caused marked enlargement of the spleen and liver, but the lungs and the kidneys remained normal. The other strain produced marked enlargement of the liver and spleen with tubercles in the kidneys. The strain which was identified as nocardia was injected into mice intraperitoneally. The mice died in three weeks with lesions in the spleen and liver. Adhesions were seen between diaphragm, spleen, and liver. Control animals inoculated with H37Rv developed progressive tuberculosis in the lungs, liver, and spleen.

The patients who were the source of the 7 strains were then clinically reviewed. In 6 cases the chest roentgenograms revealed abnormalities that were found to be consistent with the diagnosis of tuberculosis. In one case the chest film was normal. Hospitalization in this case had been based on a suspicious "positive" culture[4] for *M. tuberculosis*. The patient had received isoniazid-PAS treatment since admission to the hospital. After many subsequent sputum cultures had been negative for tubercle bacilli, a culture was isolated which again was reported as tubercle bacilli. This one culture was subjected to investigation by the methods of the present study and was identified as a strain of acid-fast bacilli.

Discussion

Both cyanogen bromide methods were used in the identification of 26 unknown strains distributed by the Sunmount Cooperative Study. As mentioned above, 3 strains were identified as human tubercle bacilli. They were specified later as H37Rv, H37Ra, and R1Rv, the last 2 of which are attenuated and could not have been verified as human tubercle bacilli by animal inoculation.

The Ravenel strain of tubercle bacilli of bovine origin (isoniazid-resistant) and some photochromogens grown in the dark are often difficult to differentiate from human tubercle bacilli just by their cultural appearance. In these situations the niacin test gives a quick and accurate differentiation.

The direct niacin test alone was chosen as the method in the second study to be discussed. As seen in table 2, 44.3 per cent of the 663 routine sanatorium cultures required subculture because of insufficient growth on the original culture.

Subculture of these strains yielded enough material in 209 cultures. The remaining 12.8 per cent, which did not yield sufficient subculture growth, proba-

[4] This culture was not available for study.

bly comprised over-aged strains or strains from patients under drug treatment. The diversified material obtained from the nine tuberculosis hospitals in this pilot study made a close analysis of this point difficult.

The focal point of the study of these 663 routine cultures was to search for atypical acid-fast bacilli in cases diagnosed as "tuberculosis" in the daily routine of the ordinary hospital laboratories. Seven such cases (1 per cent) were found. On subculturing the bacilli of the patients, their chromogenic nature could be brought out. One strain of these 7 was identified as nocardia. Nocardia is acid fast and sometimes difficult to differentiate from human tubercle bacilli on culture medium; however, by the negative niacin test it can be distinguished from human tubercle bacilli. In all 7 cases animal experiments followed. All guinea pig inoculations were negative. Three strains showed some pathologic changes after inoculation of mice. Other animal experiments confirmed that these 7 strains were not tubercle bacilli. It should be mentioned that a negative guinea pig inoculation does not exclude the possibility of infection with human tubercle bacilli which are isoniazid-resistant–catalase-deficient strains. Such strains may not cause progressive disease in the guinea pig, but they give a positive niacin test and can thus be identified as human in origin (2).

Summary

Using the production of niacin as an index, two studies were carried out: "Twenty-six unknown strains" of the Sunmount Cooperative Study were submitted to the present investigators for identification. Three of the strains were correctly found to be human tubercle bacilli and the remaining 23 strains were found to be various other strains of mycobacteria. Among 663 routine cultures collected from nine tuberculosis hospitals in New England and classified as tubercle bacilli in their respective laboratories, 6 strains proved to be mycobacteria other than human tubercle bacilli, and one strain was identified as nocardia.

Sumario

Diferenciación de los Bacilos Tuberculosos Humanos de los Bacilos Acidorresistentes Atípicos. Parte II. Aplicación Clínica

Aplicando la "prueba de la niacina," se llevaron a cabo dos estudios. A los investigadores se les entregaron para identificación "26 cepas incógnitas" del Estudio Cooperativo de Sunmount. Tres de las cepas se descubrió que eran realmente bacilos tuberculosos humanos y las 23 restantes eran otras varias cepas de micobacterias. Entre 663 cultivos corrientes recogidos de nueve sanatorios de Nueva Inglaterra y clasificados como bacilos tuberculosos en los respectivos laboratorios, 6 resultaron ser micobacterias distintas del bacilo tuberculoso humano y una cepa fué identificada como Nocardia.

Resume

Différenciation du bacille tuberculeux humain des bacilles acido-résistants atypiques. II. Application clinique

Deux études utilisant le "niacine test" ont été effectuées: "Vingt-six souches inconnues" de l'Etude coopérative de Sunmount furent soumises à l'investigation des auteurs pour identification. Trois de ces souches ont été correctement identifiées comme étant des bacilles

tuberculeux humains, les 23 autres étaient des mycobactéries de souches diverses. Parmi 663 cultures classiques recueillies dans 9 sanatoria du New England, et qui avaient été classifiées comme cultures de bacilles tuberculeux dans les laboratoires respectifs, il a été démontré que dans 6 d'entre elles les cultures se composaient de mycobactéries autres que le bacille tuberculeux. L'une des souches a été identifiée comme étant une Nocardia.

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LUNG DENSITY AS A MEASURE OF TUBERCULOUS INVOLVEMENT IN MICE[1]

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Introduction

The importance and increasing popularity of mice as laboratory animals in tuberculosis research need no documentation. Although their competition with the traditionally used guinea pig may be due partially to chemotherapy discoveries, large-scale drug screening, and a resulting stimulus to re-examine the physiology of tuberculosis, it stems mostly from the much greater rapidity and economy which these animals bring to experiments using them (1, 2). In the majority of these experiments the most important procedure is to measure the progress of tuberculosis in the mouse. Survival times (2–9) bacterial culture-counts of homogenized organs (10–14), examination of gross pathology (7, 8, 13, 16, 17), microscopic observations (13, 17–19), weighings of the entire mouse (15, 20, 26) or of its organs (2, 12, 21), and observation of externally visible lesions (16, 22) have been employed for this purpose. Only the first two are used widely as definitive methods; the third is a popular supplementary method.

Ideally, a method for evaluating mouse tuberculosis should be objective and accurate and should give reproducible results. It should reflect the degree of disease truthfully and be influenced little by infection-immunity, increasing animal age, and other yet poorly understood disease-resistance factors; therefore, it must permit short-term experiments. Finally, it should require only modest technical skill and experience. No currently used method fulfills all of these conditions. For example, although survival rates provide objective data, they seldom are determined satisfactorily within less than three weeks after challenge infection. Experiments cannot be terminated until all mice have died or until a wide enough difference between control and test groups has developed to make statistical evaluation unnecessary.

Objective measurements also are obtained when organs of sacrificed mice are homogenized, diluted, and cultured for infecting bacilli; but this method requires considerable skill, materials, apparatus, and working time. Moreover, results are not available until bacilli seeded to the test medium have produced visible colonies, something which commonly takes at least two weeks' incubation at 37°C.

Macroscopic evaluation of gross organ tuberculosis is easy and fast, but it should be used only to supplement other data because it is subjective and, therefore, no more accurate than the skill and patience of the evaluator can make it.

A new technique having all the advantages named for the hypothetically ideal

[1] From the University of Colorado School of Medicine and the Colorado Foundation for Research in Tuberculosis, 4200 East Ninth Avenue, Denver, Colorado.

method has been developed, tested, and put to routine use satisfactorily. It consists of determining the specific gravity, or density, of entire mouse lungs dissected free from the animals to be tested. The extent of pulmonary tuberculosis accurately reflects the state of tuberculous disease in intravenously infected mice (19, 21, 25, 26) and, as will be shown, the extent of pulmonary tuberculosis in turn is mirrored by lung density.

Materials and Methods

Mice: Four- to eight-week-old mice of the CF_1 strain, bred in this laboratory, were housed during each experiment in glass jars in groups of 5 or 6, and were allowed as much Purina Fox Chow-Checkers and water as they wished. The weight of the animals was distributed equally among various groups.

Vaccination: The mice were immunized with two intraperitoneal injections given a week apart, each consisting of 0.1 ml. of a water-oil emulsion and containing 0.5 mg., wet weight, of living *Mycobacterium tuberculosis*, strain H37Ra.[2] These bacilli, freshly harvested from the surface of International Union Against Tuberculosis medium egg slants (23) after two to four weeks' growth at 37° C., were ground in a Ten Broeck tissue grinder with physiologic phosphate buffer of pH 7.4. Five parts of this suspension were emulsified with ten parts of light mineral oil and two of Arlacel A[3] by repeated mixing with a syringe and large needle. One-tenth milliliter of the final emulsion contained 0.5 mg. of bacilli. Nonimmunized control animals usually were injected with the same volume of water-oil emulsion containing no antigen.

Infection: One week after the second vaccination, all animals in an experiment were infected intravenously with 0.2 ml. of physiologic phosphate buffer, pH 7.4, suspending the Erdman strain[4] of human tubercle bacilli. These had been cultivated for ten days on synthetic surface medium, generally Kirchner containing one per cent dextrose in addition to the usual ingredients (24). The number of infecting bacilli was varied according to their virulence and to the purpose of an experiment.

The infecting suspension was formed by vigorously grinding a small, weighed amount of filter paper-blotted bacillary pellicle in a Ten Broeck tissue grinder with about 5 ml. of buffer. The resulting fine suspension was diluted with more buffer to make the desired wet weight bacillary concentration per milliliter. Somewhat more than would be required to inject all of the mice in an experiment was transferred to a vaccine bottle which was then capped with a gum rubber stopper. Two doses at a time, i.e., 0.4 ml., were withdrawn through the stopper with the 0.5 ml. capacity tuberculin syringe and 26-gauge needle used for infection, and 0.2 ml. was injected into each mouse through one of the lateral tail veins. Before the next two doses were withdrawn, the vaccine bottle was shaken gently to retain suspension uniformity from infection to infection. Portions of groups were infected alternately rather than entire groups.

In one experiment (table 2), virulent Erdman bacilli were replaced, for infection, by a fast-growing nonpathogenic mycobacterium morphologically similar to *M. tuberculosis*.

Density solutions: Tuberculous lungs sink in fluids of low density which are able to float healthy lungs. Because the density of mouse lungs, healthy or tuberculous, is less than that of water, test baths are made by an appropriate mixture of organic solvents. The proportions and kinds of solvents used for experiments herein reported are indicated in table 1. Any of various organic solvents could be used, however, if mixed according to the

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[2] Obtained from Dr. William Steenken, Jr., Trudeau Laboratories, Trudeau, New York.

[3] Mannide monooleate, kindly supplied by the Atlas Powder Company, Wilmington, Delaware.

[4] Obtained from Dr. Sidney Raffel, Department of Medical Microbiology, Stanford, California.

TABLE 1

PROPORTIONS OF VARIOUS ORGANIC SOLVENTS REQUIRED TO MAKE TEST BATHS OF VARYING SPECIFIC GRAVITY

Density	n-Heptane	Ether	Methanol	Benzene	Carbon Tetrachloride
gm/ml					
0.68	100				
0.70	33	67			
0.72		89	11		
0.74		67	33		
0.76		44	56		
0.78		22	88		
0.80			100		
0.82			75	25	
0.84			50	50	
0.86			25	75	
0.88				100	
0.90				97	3
0.92				94	6
0.94				91	9
0.96				88	12
0.98				85	15
1.00				82	18

TABLE 2

EFFECT UPON MOUSE LUNG DENSITY OF INTRAVENOUS INJECTION OF 1 MG. OF FAST-GROWING SAPROPHYTIC MYCOBACTERIA

Group	Lung Densities	Mean	"t" Value
Test.....	0.73, 0.73, 0.71, 0.71, 0.71, 0.73, 0.75, 0.73, 0.71, 0.71, 0.69, 0.73, 0.73, 0.69, 0.71, 0.69	0.716	1.96
Untreated....	0.71, 0.67, 0.71, 0.71, 0.69, 0.67, 0.71, 0.69	0.695	

following formula:

$$ax + (100 - x)b = 100c$$

where

a = density of first fluid
b = density of second fluid
c = density desired
x = ml. of *a* required
100 − x = ml. of *b* required

Satisfactory [illegible]tion is available with baths increasing in density by increments of 0.02 gm. per m[illegible]th[illegible]ut having too many for convenience. Being volatile, the baths are kept in corked bottles until used. If they contain low boiling point solvents, such as ethyl ether, they must also be refrigerated.

The following description, primarily concerned with immunity experiments, mentions some conditions appropriate to these experiments but not necessarily proper for others to which this method can be applied. Some exceptions are considered in the Discussion.

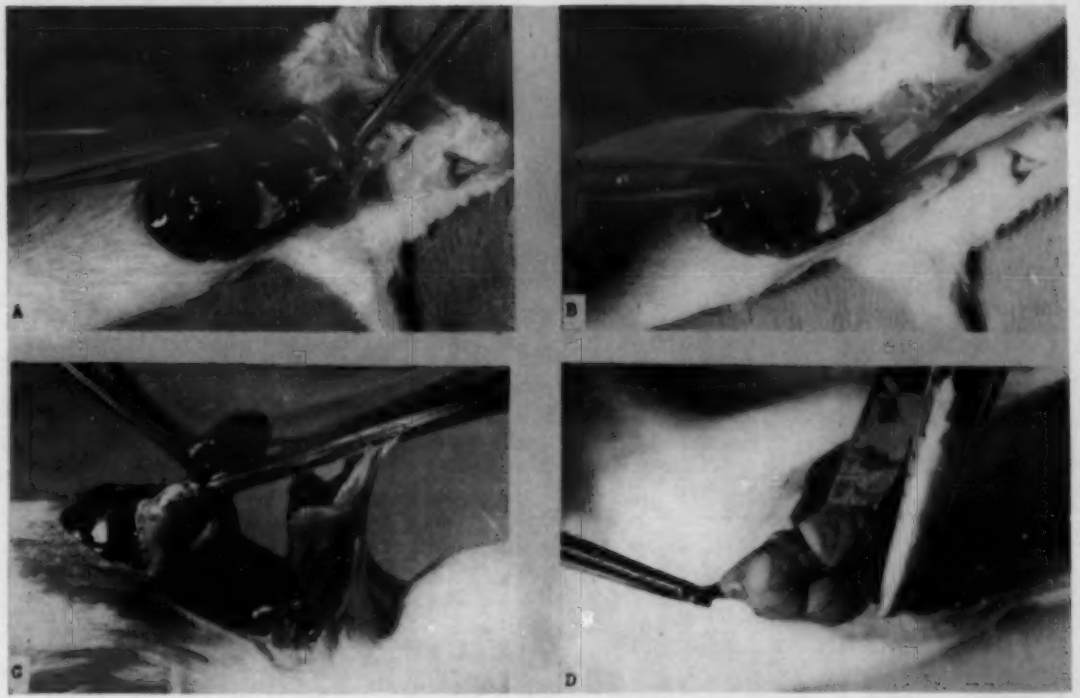

FIG. 1. Extraction of mouse lungs for subsequent density determinations. First, the anterior surface of the thorax is cut free and lifted upward (*A*). Then the lungs are pulled outward by their root while anterior connections to the body are severed (*B*). The heart is cut off (*C*) and the remaining body connections are snipped (*D*).

Challenge infection should be neither too severe, lest it overwhelm the partial, desired vaccination-resistance, nor so mild that the infection period need last more than three weeks. Infecting doses potentially able to kill unprotected animals within three to five weeks have proved ideal. When the potency of an infection is not known exactly, the experiment is best terminated as soon as unimmunized control animals just begin to show definite, overt signs of tuberculosis.

Shortly before the mice are to be sacrificed, each of seventeen 100 ml. beakers, arranged in a row and marked with the density of the bath it is to contain, is partially filled with about 60 ml. of a density bath. As many as 10 mice at a time are sacrificed with ethyl-ether vapor, and the lungs of each are dissected free, as depicted in figure 1, for testing. Extraneous tissue which remains on each mouse lung after the heart has been removed has no practical effect on subsequent density measurements because proportionally it is little and about the same for each mouse.

The removed lungs are rolled around gently on absorbent paper to remove blood and then dropped successively into the density baths, starting with the lowest and proceeding upward until the one is found which floats them. Excess fluid from each bath is blotted lightly from the lungs before they are dropped into the succeeding bath. The mean of the densities of the last bath to sink the organ and the first to float it is the estimated lung density. For example, if the lungs sink at 0.80 gm. per ml. but float at 0.82 gm. per ml., their density is recorded as 0.81 gm. per ml.

One set of density baths should be used to test no more than perhaps a hundred lungs. Once used, the baths should be autoclaved and discarded.

Experimental Results

That pulmonary tuberculosis should cause a proportional increase in lung density is so logical as to be taken for granted. Probably, this is the principal reason that the subject has hitherto been ignored.

To provide experimental proof for this hypothesis, 22 mice were infected intravenously with 0.25 mg., wet weight, of Erdman bacilli from a ten-day Sauton medium surface culture. Three days after infection and every third day thereafter, 3 mice were sacrificed and their lung densities determined. In addition, each set of lungs also was graded macroscopically on a 0 to 10 scale of increasing severity of disease before density was determined. The tip of the lower left lobe of each lung was also cut off and crushed between glass slides, stained by the Ziehl-Neelsen method, examined microscopically, and graded on a 0 to 10 basis by bacillary distribution. All measurements were made independently of each other. The experiment was terminated on the twenty-second day after infection when one mouse was found dead of tuberculosis and the others were in great distress.

The results from this experiment, plotted in figure 2, demonstrate that lung density does indeed serve as a measure of mouse tuberculosis. The three methods of measurement agree rather closely but, because only a very small portion of the entire lung was examined microscopically, this method has given somewhat erratic results.

Entire normal mouse lungs weigh approximately 0.68 to 0.70 gm. per ml. The present experiment discloses that mice about to die of tuberculosis have lung densities approaching that of water, i.e., as much as 0.97 gm. per ml. This finding has been corroborated by subsequent experiments. The lungs of mice dead for some time may be denser than water; those of the mouse found dead of

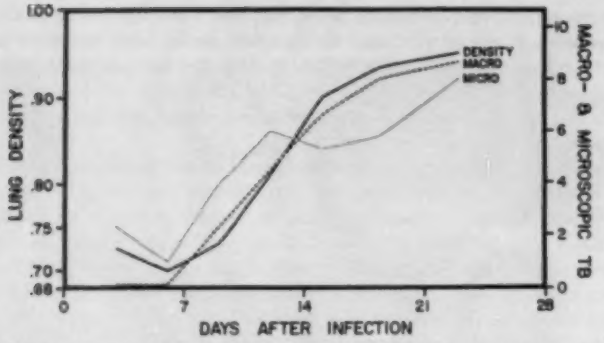

FIG. 2. Comparative evolution of pulmonary tuberculosis in mice as measured independently by lung density, and macro- and microscopic estimation. Three mice were sacrificed every third day after infection. Hence, each plotted point represents the mean of three values. Experimental details are given in the text.

tuberculosis on the last day of this experiment weighed 1.01 gm. per ml. Herein lies the reason for determining lung densities only for freshly killed mice.

In this and other experiments reported in this paper, careful efforts have been made to exclude the presence of nontuberculous pulmonary infection which might cause lung density to increase despite the fact that none among the hundreds of mice used in this laboratory have ever been found ill from spontaneous infection. Portions of lungs with increased density were smashed between two glass slides, Ziehl-Neelsen-stained, and examined under the microscope. If, after examination of such a slide, doubt remained regarding the cause for increased lung density, the second of the two slides used to smash the tissue was Gram-stained and examined. This second stain had to be used only on some mice which had been infected shortly before they were examined at autopsy and in which lung density was slightly above normal. In experiments not to be reported here this rise was found to be temporary and probably due to tuberculin allergy. All mice were carefully observed for sickness for two weeks before they were committed to an experiment.

Intravenously injected nonpathogenic mycobacteria might cause a progressive lung density increase, voiding this as a measure of tuberculosis. On the other hand, if they did not, then the method could be suggested for practical and simple quantitation of the virulence of various mycobacterial strains or species. To investigate this problem, 16 mice were injected intravenously with a large number (1 mg., wet weight) of mycobacteria, species undetermined, from a forty-eight-hour Sauton medium surface pellicle. These physically closely resemble tubercle bacilli but reproduce much more rapidly and are nonpathogenic for guinea pigs.

The mice were sacrificed forty-four days later and their lungs were examined macroscopically and by the density method. All of the animals appeared normal, both outwardly and with respect to their viscera. It may be seen in table 2 that

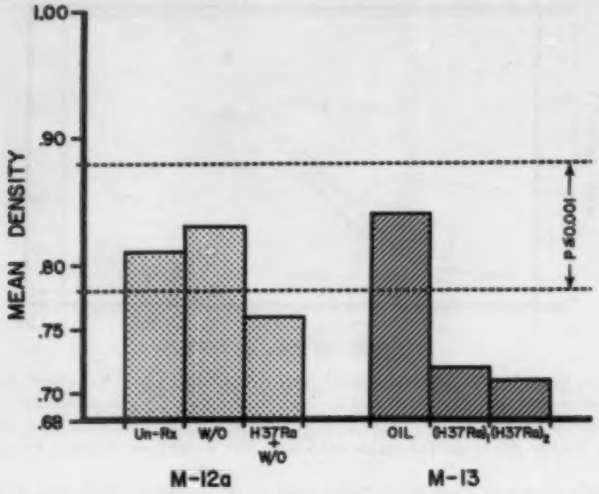

FIG. 3. Mean lung densities of immunized and nonimmunized mice in two different experiments after intravenous challenge with tuberculosis. The mice were sacrificed as soon as nonimmune animals began to show symptoms of tuberculosis. Particular attention is called to the high statistical significance of differences between group means commonly attained in lung density immunization experiments.

the mean lung density of the mice was slightly but not significantly higher than that of 8 uninfected mice killed at the same time for comparison. It would seem, then, that significant and lasting lung density increases are not induced by intravenous injection even of large numbers of nonpathogenic mycobacteria. Results from several other experiments indicated that 1 mg. of living, avirulent tubercle bacilli (H37Ra) injected intraperitoneally in 0.2 ml. of water-oil emulsion or in oil alone likewise caused no significant progressive increases.

Since rising lung density parallels progress of tuberculosis in mice infected intravenously with virulent tubercle bacilli, a comparison of mean lung densities of treated and untreated mice at various intervals or at a single interval after challenge infection should indicate the effectiveness of protective treatments. Single-interval experiments permit larger samplings since all mice are sacrificed at one time. The success of this kind of experiment for investigating acquired immunity is pointed out by figure 3.

Experiment *M-12a* in figure 3 consists of three groups of mice, one unvaccinated, a second vaccinated simply with water-oil emulsion, and the third immunized with water-oil emulsion containing living H37Ra bacilli. For experiment *M-13*, a nonimmune group was vaccinated with light mineral oil alone, and two immune groups received living H37Ra bacilli suspended in oil. In one of these $(H37Ra)_2$, mice had also been vaccinated, shortly after birth and as part of an immune-tolerance experiment, with 0.025 ml. of oil containing 0.05 mg. of living H37Ra bacilli. The animals in the two experiments were infected with different amounts of Erdman bacilli (0.15 and 0.05 mg., respectively) but were sacrificed after

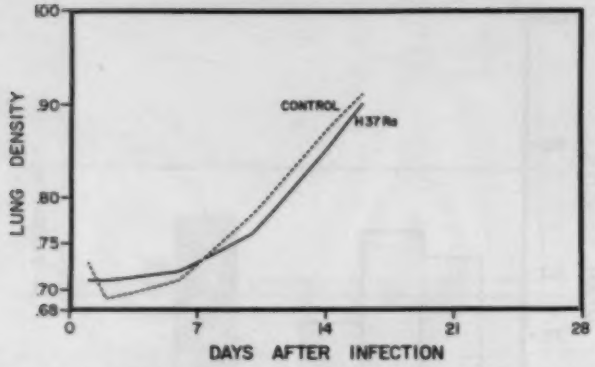

FIG. 4. The lung density method can be used to compare development of tuberculosis, with the lapse of time, in immunized and nonimmunized mice. In this particular experiment, quadruplicate samplings at four-day intervals reveal that H37Ra-induced immunity is overcome by heavy challenge infection (0.15 mg., wet weight, of Erdman human tubercle bacilli).

they had developed approximately the same degree of tuberculosis, i.e., when control mice were just beginning to show overt signs of the disease.

The high statistical significance of differences between mean lung densities of immunized and nonimmunized mice are illustrated in figure 3. This has been the usual occurrence in other immunity experiments utilizing this method. Since effective chemotherapy offers more protection than immunization against challenge infection, estimates of the potency of experimental drugs by lung density measurements should be simple to perform.

The lung density technique has, like most others, the advantage of permitting the progress of disease to be followed by periodically sampling infected mice. This and its other unique advantages, particularly its comparatively fine resolution and reproducibility, make it especially useful for quantitating infection virulence and resistance to tuberculosis. In the following two experiments, for example, the technique demonstrates strikingly the classic notion that health depends upon how well resistance balances infection.

In the first experiment, mice were immunized with a water-oil emulsion of H37Ra bacilli or were merely injected with the same kind of emulsion containing no antigen. One week after the second injection, both immunized and nonimmunized mice were infected intravenously with 0.15 mg.,[5] wet weight, of Erdman bacilli. Then, one, two, six, ten, fourteen, and sixteen days later 4 or 5 mice from each of the two groups were sacrificed, and pulmonary tuberculosis was evaluated independently both macroscopically and by lung density.

[5] By the time these two experiments were begun, the Erdman bacilli used for infection had been stabilized at a higher degree of virulence than they had shown when employed in previous experiments, a degree of virulence which they have retained to the time of this writing. Therefore, doses used in these two experiments, although comparable with each other, are not comparable by bacillary weight with those used previously.

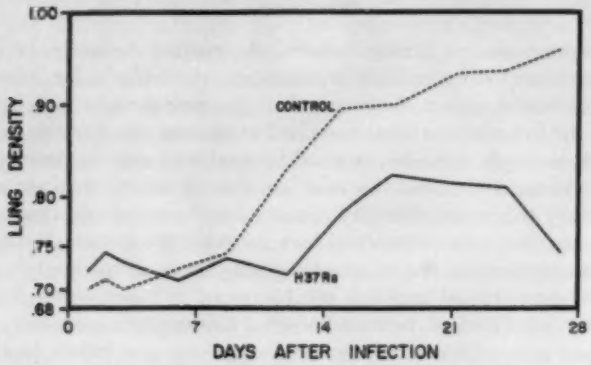

FIG. 5. Mice treated in the same way as those of figure 4 but infected with one-fifth as many tubercle bacilli (0.03 mg.) clearly show that H37Ra-immunization is effective against moderate tuberculous infection. Each point in a curve is the mean of three determinations.

In figure 4 the development of tuberculosis in both groups is depicted as assessed only by lung density; macroscopic readings are not shown, for they yielded nearly identical curves. The experiment had to be terminated somewhat prematurely on the sixteenth day, instead of the eighteenth, when the first mouse died from this heavy infection. That the degree of infection overwhelmed the degree of H37Ra-induced immunity is obvious. Since the same method of immunization has been employed successfully in numerous immunity experiments to provide immunized control animals, little doubt can exist that, had infection been lighter, immunity would have been evident. This is proved by the second experiment.

Preinfection procedures were identical in this and the preceding experiments. However, whereas mice in the first experiment were infected with 0.15 mg. of Erdman bacilli, only one-fifth of this, 0.03 mg., was injected into the mice of the second experiment. In figure 5 it is shown clearly that H37Ra-immunization is effective against less severe infection. Lung density results again were well supported by gross lung examination.

These two experiments provide additional and excellent evidence that the severity of mouse tuberculosis can be measured by lung density determinations. Density increased in direct proportion to the development of tuberculosis and lagged relative to mouse resistance and weakness of infection.

Aside from the interesting view of tuberculosis development it depicts, it is also suggested by figure 5 that immunized animals can recover from a light challenge of tuberculosis which nonimmunized animals cannot overcome. A return to normal lung density in the immunized mice commenced on the eighteenth day after infection. This leads to the plausible guess that at this time infection-immunity asserted itself and affected the subsequent course of disease. This thought calls for further investigation.

Discussion

Despite their undenied utility, none of the current methods for measuring mouse tuberculosis seems entirely satisfactory, particularly for immunity experiments. It would appear, however, that the lung density technique closely approaches the hypothetical ideal described at the beginning of this paper.

Like other methods, however, it must be used with certain precautions. The chief one is to ascertain that the mice are free of nontuberculous pulmonary disease. Usually this is not difficult because other bacterial infections commonly are more acute than tuberculosis and lack its well-known characteristics. Moreover, microscopic examination of suspected lung tissue crushed onto a glass slide and Ziehl-Neelsen-stained provides reliable proof of tuberculosis. Fortunately, carefully kept mice seldom contract bacterial diseases spontaneously.

Lungs from mice which have been dead very long give falsely high readings, presumably because of decomposition changes. Therefore, the animals should be killed only shortly before their lungs are to be tested. Although what little extraneous tissue remains attached to the lungs after the heart is removed does not affect the method's usefulness, to remove the lungs from every mouse in the same way is recommended. Individual lobes may not have the same density as the entire group; consequently, the intact lungs must be tested.

The density solutions are made with organic solvents and are not readily contaminated with lung fluids. Nevertheless, the practice in this laboratory is not to use one set of baths for more than a hundred determinations or for one experiment. Solvent evaporation, especially in mixtures containing ethyl ether, is more objectionable than water contamination in changing bath densities, but this is minimized by employing cooled baths. Although such cooling may be inadvisable for accurate determination of lung densities, it apparently did not adversely affect results in the present experiments, in which trends of the disease were the primary concern. Be that as it may, the bother of storing all stock baths in a refrigerator and using them cool can be eliminated by exclusively employing solvents of low volatility, something which is now being tried.

The risk of overinfecting moderately protected mice is pointed out in figures 4 and 5. Optimally, the challenge infection for immunization experiments should suffice to kill unprotected control animals within three to five weeks; then the experiment will have progressed far enough in two weeks to be terminated. Heavier infections might be more suitable for measuring drug potency as the degree of protection shown after one and one-half to two weeks' infection. To permit this, such drugs should offer better defense than immunization. The data in figure 5 also warn that a light infection could lead to inaccurate results in an immunity experiment lasting more than three weeks, probably from interference by infection-immunity.

Several facets of tuberculosis research have been suggested by these experiments. For example, figure 5 invites detailed investigation of the interplay between bacillary virulence and immunogenicity with respect to the lapse of time, a subject which hitherto has not been readily studied. This, in turn, suggests the

possibility of accurately quantitating the number of bacilli of a given strain which are just less than sufficient to cause progressive tuberculosis and consequently probably induce maximal infection-immunity against themselves. Subsequent investigation of infection-immunity itself would be attractive, especially because of unexpected but apparent superposition on an already existing, if perhaps incomplete, vaccine-induced immunity.

The lung density method is especially appropriate for experiments utilizing reagents available in limited quantities because these experiments can be terminated quickly and require few mice for statistical reliability. Although immunity experiments done in this laboratory routinely employ 10 mice per group, as few as 6 have been used successfully because of the small variation of results within a group.

This small variation might not be anticipated at first thought, for it would not be unreasonable to expect the amounts of air, and hence the lung densities of mice, to differ because of contrasting points in the breathing cycle at which each might have died. However, this would be contrary to what has been observed experimentally. Perhaps this low variability can be explained on the basis that lung tissue elastic recoil is stronger than either atmospheric pressure or the pressure of air inspired in the last gasp. If this is so, then the lung density method might give less reproducible results in larger animal species in which the ratio of elastic recoil to air volume is lower.

Perhaps the strongest criticism of lung density as a measure of mouse tuberculosis is that it is nonspecific, that is, an increase in lung density does not necessarily herald tuberculous infection. But, on the other hand, tubercle-like lesions on the lung, or mouse death after challenge infection with tubercle bacilli, are no more specific without at least microscopic confirmation of tuberculosis. Hence, if the mice are known to be free of nontuberculous bacterial disease, no doubt can remain, on the basis of evidence presented here and what is known about mouse tuberculosis from other research, that lung density determinations reliably measure the degree of mouse tuberculosis.

Summary

A new method for measuring mouse tuberculosis has been developed and tested. It depends upon the demonstrated fact that progression of mouse pulmonary tuberculosis is paralleled by increasing lung density. This is determined by successively dropping the entire lungs taken from ether-killed mice into organic solvent baths of increasing density until one is reached which floats the lungs. The mean of this and the preceding bath, in which they sank, is taken to be the lung density in grams per milliliter.

Virtues of this method are that it is objective, rapid, gives reliable and reproducible results, and requires little equipment or experience. The method is described in detail, and examples of its application to mouse immunization experiments are presented.

Sumario

La Densidad Pulmonar como Medida de la Invasión Tuberculosa en los Ratones

Se ha elaborado y ensayado un método nuevo para medir la tuberculosis murina. Se basa en el hecho demostrado de que el avance de la tuberculosis pulmonar en el ratón va acompañado de aumento de la densidad del pulmón. Esta se determina echando los pulmones enteros de ratones matados con éter en baños de solventes orgánicos de densidad cada vez mayor hasta encontrar un baño en que floten los pulmones. El promedio de este baño y del anterior, en que se hundieron los pulmones, se considera como la densidad pulmonar, expresada en gm. por ml.

Los méritos de este método consisten en que es objetivo, rápido, ofrece resultados fehacientes y reproducibles y requiere poco instrumental o experiencia. Se describe el método con todo pormenor y se ofrecen ejemplos de su aplicación a los experimentos de inmunización murina.

Resume

La densité pulmonaire comme mesure de l'atteinte tuberculeuse chez la souris

Une nouvelle méthode pour mesurer le degré de la tuberculose chez la souris a été mise au point et expérimentée. Elle repose sur le fait démontré de l'existence d'un parallélisme entre la progression de la tuberculose pulmonaire et l'augmentation de la densité pulmonaire chez cet animal. Ceci est déterminé en faisant successivement tomber les poumons entiers de souris tuées à l'éther dans des bains de solvant de densité croissante jusqu'à ce que l'un des bains atteigne une densité permettant aux poumons de flotter à la surface. On admet que la moyenne entre cette densité et celle du bain précédent représente la densité du poumon en g/ml.

Les avantages de cette méthode sont qu'elle est objective, rapide, fournit des résultats sûrs et reproduisibles tout en exigeant relativement peu d'instrumentation et d'expérience. La méthode est décrite en détail, et des exemples de son application dans l'immunisation de la souris sont fournis.

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THE CHEMOTHERAPEUTIC ACTIVITY UPON CHROMOGENIC MYCOBACTERIA OF CERTAIN DERIVATIVES OF THIOCARBANILIDE (SU 1906), THIAZOLINE (SU 3068), AND THIAZOLIDINONE (SU 3912)[1]

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INTRODUCTION

Within recent years, unidentified mycobacteria have been recovered from a significant number of patients obviously suffering from pulmonary tuberculosis of the lungs, bones, lymph nodes, et cetera, and evidence is accumulating which suggests that these organisms may be the causative agents of disease (1–5). The cultural characteristics of these atypical acid-fast strains are distinctly different from those of the virulent mycobacteria normally recovered from patients with active tuberculosis, but in certain respects are similar to those of the saprophytic species. In a number of instances they are apathogenic for both mice and guinea pigs, and it is therefore understandable that these strains were long considered to be saprophytic acid-fast strains or chance laboratory contaminants. Other strains, however, are nonpathogenic for guinea pigs, at the same time possessing pathogenicity for mice when relatively small infective doses are employed.

Differences in pathogenicity of these photochromogenic "yellow bacilli" are not the only reason why it may be assumed that the chromogenic mycobacteria represent a heterogenous group. Indeed, a large number of variants have been recognized which are not only differentiated from the true tubercle bacilli and the saprophytes, but also differ among themselves in certain cultural characteristics, in the development of pigment (particularly under the influence of light), in immunologic specificities, and in especially well-defined patterns of resistance to known antituberculous agents.

According to Yegian and Budd (6) and Steenken and associates (7), many chromogenic strains of mycobacterium are naturally resistant to certain substances which are normally bacteriostatic or bactericidal to *M. tuberculosis* and saprophytic mycobacteria. In view of the wide variation in reactivity of these strains to the different antituberculous compounds, it seemed of interest to investigate the susceptibility of several strains of chromogenic mycobacteria to representatives of three new classes of antituberculous compounds which have recently been synthesized and tested in the laboratories of the present writers, namely, thiocarbanilides (8–12), thiazolines, and thiazolidinones (13, 14).

[1] From the Research Department, CIBA Pharmaceutical Products Inc., Summit, New Jersey.

EXPERIMENTAL

Cultures: Nine different chromogenic strains were used in this study.[2] According to E. H. Runyon (15), they are representative of the principal types of organisms implicated in human disease and were isolated from resected human diseased lung, sputum, or gastric washings and were designated by him as follows:

P1. Photochromogen; mouse pathogenic
P3. Non-photochromogen; nonpathogenic
P4. Photochromogen; mouse pathogenic
P6. Scotochromogen; nonpathogenic
P8. Photochromogen; mouse pathogenic
P17. Non-photochromogen; nonpathogenic[3]
P18. Photochromogen; mouse pathogenic
P21. Photochromogen; mouse pathogenic
P26. Photochromogen; mouse pathogenic

Antituberculous agents used: Su 1906: 4-butoxy-4'-dimethylamino-thiocarbanilide; Su 3068: 3-(*p*-isoamyloxyphenyl)-2-(*p*-isoamyloxyphenylimino)-4-methyl-4-thiazoline-HCl; Su 3912: 3-(*p*-isoamyloxyphenyl)-2-(*p*-isoamyloxyphenylimino)-4-thiazolidinone; PAS (para-aminosalicylic acid); isoniazid; amithiozone; viomycin,[4] and streptomycin.

In vitro tests: The bacteriostatic activities of the various antituberculous agents against the nine chromogenic strains were determined at 37° C. in Kirchner's liquid medium containing 0.03 per cent Tween® 80 and 0.5 per cent bovine serum albumin, Fraction V (9). After seven days of incubation, the minimal concentrations capable of completely inhibiting the growth of the test organisms were determined. *M. tuberculosis* (H37Rv) and *M. smegmatis* (607) were used for purposes of comparison.

In vivo tests: Seven-day-old cultures of strains P1, P4, P8, P18, P21, and P26, grown in the modified Kirchner's liquid medium at 37° C., were diluted with 0.9 per cent sodium chloride solution to a density corresponding to a light transmission of 76 per cent in the Lumetron colorimeter fitted with a 589 filter. Five-tenths of a milliliter of this suspension were injected intravenously into groups of 10 CF_1 mice weighing between 16 and 20 gm. Treatment was instituted immediately after infection and was continued for thirty consecutive days, at which time all surviving mice were sacrificed and the extent of kidney and lung involvement was evaluated macroscopically. With the exception of the two antimicrobials which were administered subcutaneously, the drugs were thoroughly incorporated into finely ground Rockland diet and fed ad libitum.

RESULTS

In Vitro Experiments

As recorded in table 1, the minimal concentrations (γ per ml.) necessary to completely inhibit the growth of the nine chromogenic strains as well as that of *M. tuberculosis* (H37Rv) and *M. smegmatis* (607) vary to a considerable extent. The susceptibilities of the various chromogenic strains toward the different compounds are not only at considerable variance with that of either tubercle bacilli or the saprophyte *M. smegmatis*, but there are considerable variations among the chromogens themselves.

The *in vitro* activities of the thiocarbanilide Su 1906 and amithiozone upon chromogenic strains closely parallel each other; this is in agreement with the

[2] Kindly furnished by Dr. Ernest Runyon, Veterans Administration Hospital, Sunmount, New York.

[3] This strain was received as mouse pathogenic, but tests have failed to establish this property.

[4] Manufactured as Vinactane by CIBA Pharmaceutical Products Inc., Summit, New Jersey.

TABLE 1

IN VITRO ACTIVITIES OF VARIOUS ANTITUBERCULOUS AGENTS AGAINST NINE CHROMOGENIC MYCOBACTERIA, M. SMEGMATIS, AND M. TUBERCULOSIS

Test Organism	Minimal Concentrations Completely Inhibiting Growth (γ/ml.) of:						
	Su 1906	Su 3068	PAS	Viomycin	Streptomycin	Isoniazid	Amithiozone
P1 (pathogenic)	0.33	12.0	125.00	94.0	23	<0.25	1.00
P3 (nonpathogenic)	63.00	23.0	0.75	7.5	3	3.80	46.00
P4 (pathogenic)	2.00	125.0	63.00	23.0	15	3.00	<0.25
P6 (nonpathogenic)	15.00	15.0	15.00	15.0	15	1.00	15.00
P8 (pathogenic)	0.75	12.0	4.00	94.0	46	0.75	0.50
P17 (nonpathogenic)	>125.00	30.0	0.75	7.5	2	2.00	63.00
P18 (pathogenic)	2.00	63.0	63.00	30.0	23	11.00	<0.25
P21 (pathogenic)	2.00	63.0	125.00	23.0	15	2.00	<0.25
P26 (pathogenic)	2.00	63.0	30.00	23.0	>125	2.00	<0.25
M. smegmatis #607	>125.00	23.0	>125.00	15.0	4	3.00	46.00
M. tuberculosis H37Rv	2.00	7.5	<0.25	4.0	1	<0.25	<0.25

previous obeservations of the present writers that cross-susceptibility (or cross-resistance) exists between these two compounds in the case of M. *tuberculosis* (11). Both compounds suppressed the growth of six chromogenic strains (P1, P4, P8, P18, P21, P26) and had lower activities against three other strains (P3, P6, and P17). A certain relationship exists apparently between mouse pathogenicity of chromogenic strains and susceptibility to Su 1906 and isoniazid: Most pathogenic strains were susceptible to both substances, although this susceptibility to either compound is not necessarily coupled in all instances. One strain, for instance P18, was considerably more susceptible to Su 1906 and the chemotherapeutically related amithiozone than to isoniazid. The three nonpathogenic strains were very susceptible to isoniazid but were much less responsive to the thiocarbanilide Su 1906.

There was apparently no connection between the activities exerted by the sulfur-containing compounds and the other antituberculous substances. Streptomycin, highly active against both *M. tuberculosis* and *M. smegmatis*, had comparable activity only against two chromogens (P3 and P17), but only moderate activity against the seven other strains. Isoniazid, on the other hand, while possessing considerable activity against tubercle bacilli and somewhat lower, but nevertheless good, activity against *M. smegmatis*, was active against eight chromogenic strains and considerably less active in one instance (P18). With one exception, however, the activity of isoniazid against chromogens was only one-tenth to one-fiftieth of that usually exerted against M. *tuberculosis* (H37Rv). Para-aminosalicylic acid, of considerable activity against tubercle bacilli, but inactive in the case of *M. smegmatis*, had very good activity against three chromogenic strains (P3, P8, and P17), and was only active or inactive against the other six strains.

The thiazoline derivative Su 3068 had low activity against all mycobacteria;

its activity against three chromogenic strains (P1, P6, P8), however, was comparable to that exerted against *M. tuberculosis*.

According to these *in vitro* results, the nine chromogenic strains may be classified into five groups of distinct patterns with respect to their susceptibility or natural resistance toward the different antituberculous compounds tested.

Pattern 1: High susceptibility to Su 1906, amithiozone, and isoniazid, but resistance to PAS, streptomycin, and viomycin (strains P1, P4, P21, and P26).

Pattern 2: High susceptibility to Su 1906, amithiozone and isoniazid; but, in addition, a fair degree of susceptibility to PAS (strain P8).

Pattern 3: High susceptibility only to Su 1906 and the chemotherapeutically related amithiozone, but resistance to all other compounds (strain P18).

Pattern 4: High susceptibility to PAS; to a lesser degree, to isoniazid, streptomycin, and viomycin; resistance to both Su 1906 and amithiozone (strains P3 and P17).

Pattern 5: Susceptibility only to isoniazid (strain P6).

While the virulent *M. tuberculosis* (H37Rv) is known to be highly susceptible to Su 1906, isoniazid, PAS, amithiozone, and streptomycin, and moderately susceptible to viomycin, *M. smegmatis* proved to be highly susceptible only to isoniazid and streptomycin. A certain relationship between pathogenicity and drug susceptibility could be observed among the chromogenic mycobacteria tested, in that all six pathogenic strains were highly susceptible to Su 1906, while five of the strains were also highly susceptible to isoniazid.

In Vivo Experiments

In a series of mouse experiments performed under various conditions of drug administration, the response to infections with six different chromogenic strains (P1, P4, P8, P18, P21, and P26) was compared with the results obtained with infections with *M. tuberculosis* (H37Rv). The following two experiments were performed with various antituberculous agents at concentrations which usually produce comparable therapeutic response in mice infected with *M. tuberculosis* (H37Rv).

The activities of Su 1906, Su 3068, Su 3912, viomycin, streptomycin, and isoniazid against three different infections (two chromogenic strains (P1 and P8) and *M. tuberculosis* (H37Rv)) were studied. Treatment was instituted immediately upon infection and continued for thirty consecutive days. The results are recorded in table 2. The following conclusions may be made with respect to the influence of the various drugs upon each specific infection:

Infectious agent: chromogenic strain P1: Su 1906 and Su 3068 completely suppressed the development of lesions in the kidneys and, to a considerable extent, the development of lesions in the lungs. In the case of lung involvement, however, Su 3068 proved more effective than Su 1906, as has been regularly observed in chemotherapeutic trials with *M. tuberculosis* (H37Rv) (13). Isoniazid was definitely inferior to either Su 3068 or Su 1906. Streptomycin, viomycin, and PAS were virtually without effect.

TABLE 2

IN VIVO ACTIVITIES AGAINST CHROMOGENIC STRAINS P1 AND P8 AS COMPARED TO H37Rv

Drug and Dose	Organ	Strain	Tuberculosis Index	Drug and Dose	Organ	Strain	Tuberculosis Index
Su 1906, 0.1%, diet	Kidney	P1	0.00	Streptomycin, 3 mg. per 20 gm. per day subcutaneously	Kidney	P1	0.80
		P8	0.66			P8	0.55
	Lung	P1	1.05		Lung	P1	2.20
		P8	3.10			P8	3.70
		H37Rv	1.00			H37Rv	2.50
Su 3068, 0.1%, diet	Kidney	P1	0.00	PAS, 1.0%, diet	Kidney	P1	1.90
		P8	0.55			P8	1.10
	Lung	P1	0.40		Lung	P1	3.90
		P8	3.00			P8	3.80
		H37Rv	0.55			H37Rv	3.00
Su 3912, 0.1%, diet	Kidney	P1	0.40	Isoniazid, 0.1%, diet	Kidney	P1	0.00
		P8	0.44			P8	0.28
	Lung	P1	1.25		Lung	P1	1.50
		P8	3.90			P8	2.50
		H37Rv	0.60			H37Rv	0.45
Viomycin, 3 mg. per 20 gm. per day subcutaneously	Kidney	P1	0.90	Controls (untreated)	Kidney	P1	2.00
		P8	1.10			P8	2.60
	Lung	P1	2.80		Lung	P1	4.00
		P8	3.70			P8	3.70
		H37Rv	2.90			H37Rv	4.00

The *organ involvement* is the calculated average degree of gross pathologic involvement; thus, an index of 0 signifies the absence of lesions, while an index of 4 indicates maximal involvement.

Infectious agent: chromogenic strain P8: Although the pathogenicity of strain P8 for mice was approximately equal to that of strain P1, the protection afforded by the various therapeutic agents was considerably less in the mice infected with strain P8 than in those infected with strain P1. All drugs tested suppressed the development of kidney lesions, with isoniazid slightly superior to Su 3068, Su 1906, Su 3912, and streptomycin; however, none was capable of arresting, to any significant degree, the development of lung lesions. This relative resistance of strain P8 to chemotherapeutic agents is noteworthy since this strain, in the *in vitro* assay, was only slightly less susceptible than strain P1 toward the various agents. Thus, contrary to the results obtained with strain P1, no parallel exists between *in vitro* and *in vivo* results in the case of strain P8.

Infectious agent: M. tuberculosis (H37Rv): Under the conditions of this experiment, the activities of Su 3068 and Su 3912 in mice infected with tubercle bacilli

were comparable with the activity of isoniazid; Su 1906 proved to be slightly less active. Viomycin, streptomycin, and PAS had little effect upon the development of lung lesions, despite the fact that they extended considerably the survival time of the infected mice; this is in accord with the results of numerous experiments previously performed in the laboratories of the present investigators. In similar experiments with the chromogenic strains P4, P18, P21, and P26, Su 3068 had greater activity against strains P21 and P26, equal activity against strain P4, and slightly less activity against strain P18 when compared with isoniazid. In all four types of infections, its activity was slightly superior to that of Su 1906. Streptomycin and viomycin were consistently inferior, while PAS was virtually without activity. Consequently, of all six strains tested, strain P8 was the most resistant toward the various antituberculous compounds; whereas strains P1 and P18 were the least resistant. The three other strains (P4, P21, and P26) were comparable with *M. tuberculosis* (H37Rv).

In further experiments with strain P1, the relative activities of the different

TABLE 3

GRADED DOSE RESPONSE IN MICE INFECTED WITH CHROMOGENIC STRAIN P1

Drug	Index of Gross Lung Involvement and Per Cent Survivors After 30 Days of Treatment at Indicated Doses in Diet:				
	0.1%	0.05%	0.025%	0.01%	0.005%
Su 1906	1.10 (100)	1.70 (100)	3.0 (100)	3.1 (100)	4.0 (50)
Su 3068	0.60 (100)	0.65 (100)	3.1 (100)	3.5 (100)	3.9 (100)
Su 3912	2.80 (100)	3.00 (100)	3.8 (80)	4.0 (80)	4.0 (60)
Isoniazid	0.55 (100)	1.10 (100)	1.4 (100)	4.0 (90)	4.0 (90)

Untreated controls: 4.0 (75 controls).

Figures in parenthesis: per cent survival. The others are tuberculosis factors for lung involvement.

TABLE 4

EFFECT OF DELAYED THERAPY ON LUNG INVOLVEMENT IN MICE INFECTED WITH CHROMOGENIC STRAIN P1

Between Time of Infection and Start of Therapy	Index of Gross Lung Involvement After 30 Days at Indicated Doses in Diet				
	Su 1906 0.1%	Su 3068 0.1%	Su 3912 0.1%	Isoniazid 0.1%	Untreated Controls
days					
0	0.8	0.3	2.4	0.4	4.0
3	1.9	0.7	2.3	0.6	4.0
6	1.8	1.7	2.9	1.3	4.0
9	2.8	2.6	3.4	2.2	4.0
12	2.9	2.5	3.0	2.6	4.0
15	3.1	3.0	3.3	2.8	4.0

compounds were more quantitatively assessed. The lower limits of activity against strain P1 for Su 1906, Su 3068, Su 3912, and isoniazid were determined. The results, which are summarized in table 3 and expressed as indices of gross lung involvement and per cent survival, indicate, in the case of strain P1, that Su 3068 and isoniazid exerted similar activities; whereas, Su 1906 was slightly, and Su 3912 decidedly, less active. Delayed therapy with the different drugs started at various intervals of time following infection indicated that Su 3068 and isoniazid exerted identical degrees of activity; Su 1906 was slightly less effective; while Su 3912 had almost no activity (table 4).

Discussion

In the present study it has been shown that chromogenic mycobacteria are susceptible, *in vitro* and *in vivo*, to certain derivatives of the thiocarbanilide, thiazoline, and thiazolidinone series. Wolinsky and associates (5), who have examined the *in vitro* drug susceptibility of three chromogenic strains isolated from human tuberculosis, reported that two of these strains were as susceptible to amithiozone and Su 1906 as the H37Rv strain, while the third strain was definitely more resistant. The nine strains used in the present study have shown definite patterns of *in vitro* resistance and susceptibility to a number of known antituberculous agents. The objection may be raised that these strains may have been isolated from subjects treated with isoniazid, streptomycin, PAS, or combinations of these agents. None of these strains was found to be resistant to isoniazid, with the possible exception of strain P18. There were, however, several strains with considerable resistance to streptomycin. Consequently, it must be borne in mind that still other patterns of drug susceptibilities may exist with other strains which have had no contact with these antituberculous agents. The *in vivo* susceptibility of these strains, however, did not correspond in all instances to these patterns. In mice infected with chromogenic strains P1, P21, and P26, Su 3068 proved slightly superior to Su 1906 and isoniazid, while the latter compound was slightly superior in mice infected with strains P4, P8, and P18. The high susceptibility of these strains toward thiocarbanilides and thiazoline-derivative Su 3068 is quite surprising. This is true particularly in view of the natural resistance these chromogens possess toward known, effective antituberculous agents, which in general are superior, *in vitro* and *in vivo*, to the thiocarbanilides and thiazolines against the various strains of tubercle bacilli. Of interest was the resistance of most chromogenic strains toward PAS, which is highly active *in vitro* against the H37Rv strain.

The wide limits within which susceptibility and resistance to most known antituberculous compounds did vary may indicate that this group of chromogenic mycobacteria, although associated with human atypical tuberculosis, may indeed be as heterogenous as the group representing the common, saprophytic mycobacteria. A new variant of *M. tuberculosis* (H37Rv) has been artificially produced in these laboratories. This not only demonstrated an acquired resistance to the thiocarbanilides, namely Su 1795 and 1906, against which the original strain was highly susceptible, but also had altered morphologic and cultural

characteristics. These might have justified the inclusion of this strain in a group intermediate between the true tubercle bacilli and the saprophytic species had its origin not been known.

If it were true that the group of chromogenic mycobacteria is composed of a number of different species, variants or mutants, such a heterogenicity would adequately explain the great variations in resistance to numerous antituberculous agents.

It is well known that, within the class of mycobacteria, individual species display a wide range of susceptibility toward the various antituberculous compounds. While, for instance, *M. tuberculosis* is highly susceptible to isoniazid, the lepra bacillus and actinomyces apparently possess pronounced natural resistance toward this drug. In the case of thiocarbanilides, tubercle bacilli are less susceptible to Su 1906 than to isoniazid, while, in the case of the actinomyces and nocardias, the susceptibilities are reversed (16). The lepra bacillus is similarly more susceptible to thiocarbanilides than to isoniazid, as may be concluded from the favorable therapeutic results obtained with Su 1906 in human leprosy (17).

Although infections caused by chromogenic mycobacteria are present only in a small percentage of the over-all incidence of tuberculosis, their natural resistance to many of the commonly used tuberculostatic agents constitutes a serious therapeutic problem. For this reason the pronounced susceptibility of many of these strains to thiocarbanilides, and in particular toward the thiazoline derivative Su 3068, may provide a new and more effective therapeutic approach to certain cases of atypical tuberculous infections.

Summary

The *in vitro* drug susceptibility of nine chromogenic strains and the *in vivo* response of six of these strains toward various known antituberculous agents, especially certain derivatives of the thiocarbanilide, thiazoline, and thiazolidinone series, are described. Among these compounds, Su 3068, a thiazoline, and Su 1906, a thiocarbanilide, showed surprisingly strong activity.

Sumario

La Actividad Quimioterapéutica Ejercida sobre las Micobacterias Cromógenas por Ciertos Derivados de las Tiocarbanilidas (Su 1906), las Tiazolinas (Su 3068) y las Tiazolidinonas (Su 3912)

Descríbese la susceptibilidad *in vitro* de nueve cepas cromógenas de micobacterias y la respuesta *in vivo* de seis de ellas a varios conocidos medicamentos antituberculosos, y en particular ciertos derivados de las series de la tiocarbanilida, la tiazolina y la tiazolidinona. Entre esos compuestos, el Su 3068, una tiazolina, y el Su 1906, una tiocarbanilida, revelaron una actividad sorprendente por lo poderosa.

Resume

Activité chimiothérapeutique de certains dérivés des thiocarbanilides (Su 1906), thiazolines (Su 3068), et thiazolidinones (Su 3912) sur les mycobactéries chromogènes

Description de la sensibilité *in vitro* de neuf souches chromogènes et la réponse *in vivo* de six d'entre elles vis-à-vis de divers agents antituberculeux connus, en particulier de

certains dérivés des séries thiocarbanilide, thiazoline et thiazolidinone. Parmi ces composés, Su 3068, une thiazoline, et Su 1906, une thiocarbanilide, ont fait preuve d'une activité marquée.

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THE ANTITUBERCULOUS ACTIVITIES OF SUBSTITUTED THIAZOLINES AND THIAZOLIDINONES[1]

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INTRODUCTION

In earlier communications, the antituberculous activity in experimentally infected animals of a large series of 4,4′-disubstituted thiocarbanilides was recorded (1–3). More recently, a number of thiazolines and thiazolidinones have been synthesized (4) from certain of these thiocarbanilides and their chemotherapeutic activities compared with those of the corresponding parent compounds. The results of this study are reported in the present paper. The compounds tested are represented by the structural formulas shown in table 1.

The thiazolines used in these experiments are stable in alkaline and acid alcoholic (aqueous) solutions at ordinary temperatures, but may undergo slight decomposition at elevated temperatures. Thiazolidinones, on the other hand, are sensitive to acid and alkali in aqueous alcoholic solutions; decomposition is slow at room temperature but proceeds at a rapid rate at elevated temperatures. Upon basic hydrolysis, the thiazolidinone ring of the Su 3912 type is opened, yielding thioglycollic acid as one of the products.

METHODS

In vitro tests: All compounds were evaluated for *in vitro* activity against *M. tuberculosis* var. *hominis* (H37Rv) in a modified Kirchner's liquid medium[2] containing 0.03 per cent Tween® 80 and 0.5 per cent serum albumin. The test organism was grown in this medium for seven days at 37° C. and the resulting culture was then diluted with Kirchner's medium (without serum albumin) to contain approximately 50,000 organisms per ml. Inocula of 0.1 ml. amounts of diluted culture were used to seed 10 ml. of broth containing various concentrations of the test substance. All initial (concentrated) solutions of test substances, as well as subsequent dilutions in broth, were sterile. All assay tubes were incubated for fourteen days and the minimal inhibiting concentrations were determined.

In vivo tests: Mice and guinea pigs: The procedure employed was identical to that described in detail in a previous communication (2). In mice, the activity of various compounds at the doses tested was measured by the increase in T50 values calculated for the medicated groups over those obtained for the control (untreated) groups. An increase in

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[1] From the Research Department, CIBA Pharmaceutical Products Inc., Summit, New Jersey.

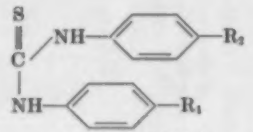
[2] Composition of modified Kirchner's medium:

$Na_2HPO_4 \cdot 7H_2O$	3.0 gm.	Asparagin	5.0 gm.
KH_2PO_4	4.0 gm.	Iron ammonium citrate	0.05 gm.
$MgSO_4$ (anhydrated)	0.6 gm.	Glycerin	20.0 ml.
Sodium citrate	2.5 gm.	Tween® 80	0.3 ml.
		Distilled water, *quantum sufficit*	1,000 ml.

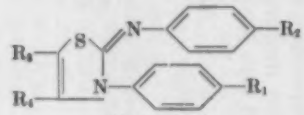
Autoclave for fifteen minutes at 15 pounds. Sterile (Seitz-filtered) serum albumin is then added.

TABLE 1

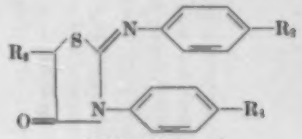
THIAZOLINES AND THIAZOLIDINONES YIELDING AN INCREASE IN T50 OF MORE THAN 15 DAYS, AND CORRESPONDING CONCENTRATIONS IN THE DIET



(*Thiocarbanilide*)



(*Thiazoline*)



(*Thiazolidinone*)

Compound Number	R_1	R_2	R_4	R_5	*In Vitro* Minimal Inhibiting Concentration	Minimal Active Dose in Diet
			A. *Thiazolines*			
					γ/*ml.*	*per cent*
Su 3174	$-nC_4H_9$	$-nC_4H_9$	—H	$-CH_3$	>32	0.100
Su 3170	$-OC_2H_5$	$-OC_2H_5$	—H	piperidinoethyl	125	0.100
Su 3168	$-OC_3H_7$	$-OC_3H_7$	—H	$-CH_3$	4	0.050
Su 3131	$-OC_3H_7$	$-OC_3H_7$	—H	piperidinoethyl	12	0.300
Su 3295	$-OnC_5H_{11}$	$-OnC_5H_{11}$	—H	$-CH_3$	>125	0.300
Su 3068	$-OiC_5H_{11}$	$-OiC_5H_{11}$	—H	$-CH_3$	16	0.010
Su 3191	$-OiC_5H_{11}$	$-OiC_5H_{11}$	—H	$-C_6H_5$	32	0.100
Su 3130	$-OiC_5H_{11}$	$-OiC_5H_{11}$	—H	piperidinoethyl	24	0.100
Su 3183	$-OiC_5H_{11}$	$-OiC_5H_{11}$	$-CH_3$	$-CH_3$	12	0.100
Su 4260	$-OiC_5H_{11}$	$-OiC_5H_{11}$	—H	$-C(CH_3)_3$	>125	0.050
Su 4268	$-OCH_2CH_2CH(CH_3)CH_2CH_3$	$-OCH_2CH_2CH(CH_3)CH_2CH_3$	—H	$-CH_3$	>125	0.100
Su 3117	$-OCH_2CH{=}CH_2$	$-OCH_2CH{=}CH_2$	—H	$-CH_3$	4	0.100
Su 3132	$-OCH_2CH{=}CH_2$	$-OCH_2CH{=}CH_2$	—H	piperidinoethyl	24	0.100
Su 4197	$-OC_4H_9$	$-OCH_2CH_2N(C_2H_5)_2$	—H	$-CH_3$	32	0.050
Su 4183	$-OiC_4H_9$	$-OCH_2CH_2N(C_2H_5)_2$	—H	$-CH_3$	24	0.025
Su 4161	$-N(CH_3)_2$	$-OC_4H_9$	—H	$-CH_3$	>32	0.050
			B. *Thiazolidinones*			
Su 3860	$-OC_3H_7$	$-OC_3H_7$	—H		>32	0.100
Su 3912	$-OiC_5H_{11}$	$-OiC_5H_{11}$	—H		32	0.010
Su 4262	$-OiC_5H_{11}$	$-OiC_5H_{11}$	$-CH_3$		32	0.050
Su 4269	$-OCH_2CH_2CH(CH_3)CH_2CH_3$	$-OCH_2CH_2CH(CH_3)CH_2CH_3$	—H		32	0.100
Su 3830	$-OCH_2CH{=}CH_2$	$-OCH_2CH{=}CH_2$	—H		16	0.100

T50 of less than three days was not considered as indicative of activity. In the case of guinea pigs, the antituberculous activity was expressed in terms of gross involvement of lungs, liver, and spleen according to the method of Feldman and Karlson (5–6).

Results

The conversion of thiocarbanilides into corresponding thiazolines and thiazolidinones introduces additional sites for possible substitution. As indicated in the chemical structure of the thiazolines, two new positions, R_3 and R_4, are made available; while in the case of the thiazolidinones, only one new site, R_3, may be varied.[3]

In vitro tests: In those instances in which a comparison could be made of the *in vitro* activities of the thiazolines and thiazolidinones with those of their corresponding thiocarbanilides, the activity of the parent substance was generally superior.[3]

In vivo tests: Among the 41 thiazolines and 14 thiazolidinones tested, several were found to exert good antituberculous activity in that they extended the survival time of infected mice over that of the controls by more than fifteen days (T50 of 35-plus days). These active compounds are indicated in table 1.

A comparison of the *in vivo* activities of the thiocarbanilides with those of the derived substances permits several conclusions:

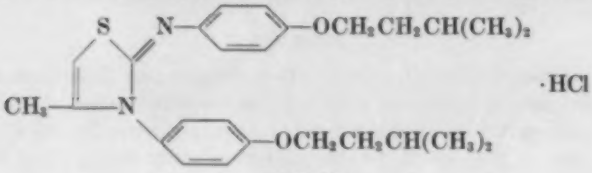
(*a*) *Thiazolines:* In five of six substances, substitution of a methyl group at position R_3 yielded derivatives with little or no activity in mice (Su 3171, Su 3184, Su 3182, Su 4149, Su 3173), although the parent thiocarbanilides were active. In the single exception (Su 3183), the resulting activity was only slightly inferior to that of its precursor. The addition of a methyl group at R_4 (R_3=H) led, at most, to only a slight loss of activity in nine of twelve derivatives (Su 3174, Su 3142, Su 3168, Su 3068, Su 4197, Su 4183, Su 4705, Su 4716, Su 4161) and to appreciable losses in three instances (Su 3117, Su 3295, Su 4174). The introduction of a piperidinoethyl group at R_4 in five thiazolines had no adverse effect upon activity.

(*b*) *Thiazolidinones:* Three unsubstituted compounds (R_3=H) retained activity (Su 3912, Su 4269, Su 3830), while a fourth compound (Su 4261) completely lost all activity. In the single instances investigated, the introduction of a methyl group (Su 4262) brought about a slight reduction of activity; while the addition of a 3-pyridinal substituent abolished all activity.

Among the thiazoline and thiazolidinone compounds examined for their antituberculous activity, two derivatives, namely, 3-(*p*-isoamyloxyphenyl)-2-(*p*-isoamyloxyphenyl-imino)-4-methyl-4-thiazoline·HCl (Su 3068) and 3-(*p*-isoamyloxyphenyl)-2-(*p*-isoamyloxyphenyl-imino)-4-thiazolidinone (Su 3912) had outstanding activities at concentrations in the diet as low as 0.01 per cent. Since these substances proved to be somewhat more effective as antituberculous agents

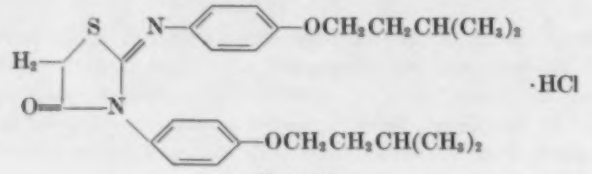
[3] Details of the *in vitro* and *in vivo* (mouse) results obtained with each of the thiazolines and thiazolidinones investigated are shown in the appendix contained in the reprint of this article.

than their corresponding thiocarbanilide (Su 1748) and, in view of the fact that their activities in mice were superior to those of two outstanding thiocarbanilides



Su 3068

(Su 1795 and Su 1906), described in an earlier publication (2), they were accordingly investigated in greater detail.



Su 3912

Su 3068 and Su 3912 are both relatively insoluble in water at pH 7.0 and above, and very sparingly soluble in acid solution; they are, however, readily soluble in ethyl alcohol and propylene glycol. Their respective melting points are 180 to 181° C. and 130 to 133° C.

In table 2, the activities of these two substances are compared with those of Su 1795 and Su 1906, which were, as already noted, the two most active compounds among 295 thiocarbanilides studied.

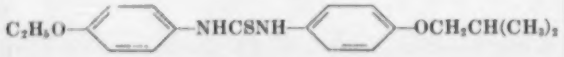
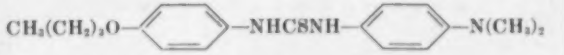
Limited and delayed-therapy experiments: In the limited therapy experiment, treatment of the infected mice, instituted immediately upon infection, was limited to periods of three, six, nine, and twelve days, respectively (table 3). In the delayed-therapy experiment, treatment was delayed for periods ranging from three to fifteen days following infection; but in each instance treatment was maintained for a total of twenty-one consecutive days (table 4). In both experiments, all surviving mice were sacrificed on the thirty-fifth day post infection, and the T50 values were calculated for each group.

According to the results obtained, it is evident that Su 3068 possesses a greater activity than Su 3912 in that it exerted an excellent protective action at a concentration of 0.05 per cent in the diet when administered for only three days following infection or when therapy, using this dose, was delayed for fifteen days. Its activity was approximately the same as that observed with isoniazid.

Further comparisons of antituberculous activities of Su 3068 and isoniazid: Since Su 3068 and isoniazid exhibited the same degree of activity, according to the criteria employed in the preceding experiments, the following experiment was

TABLE 2

COMPARATIVE ANTITUBERCULOUS ACTIVITIES OF SELECTED THIOCARBANILIDES, SU 3068 AND SU 3912

Compound	Structure	Minimal Inhibiting Concentration	Per Cent Concentration in Diet	Increase in T50 Value over Controls	Per Cent Survivors
		γ per ml.		*days*	
Su 1795		8	0.100	15.0+	100
			0.050	15.0+	70
			0.025	−1.0	0
Su 1906		4	0.050	15.0+	100
			0.025	15.0+	80
			0.010	1.0	10
Su 3068	(recorded elsewhere)	16	0.050	15.0+	100
			0.010	15.0+	90
			0.005	1.5	0
Su 3912	(recorded elsewhere)	32	0.050	15.0+	100
			0.010	15.0+	70
			0.005	2.5	30

Note: The activities recorded for Su 1795 and Su 1906 were taken from a previous communication (2).

TABLE 3

EFFECT OF LIMITED THERAPY WITH SU 3068, SU 3912, AND ISONIAZID ON MOUSE TUBERCULOSIS (H37Rv)

Compound	Concentration in Diet	Duration of Treatment	Increase in T50	Per Cent Survivors
	per cent	*days*	*days*	
Su 3068	0.05	3	15+	60
		6	15+	80
		9	15+	100
		12	15+	100
Su 3912	0.05	3	3.0	40
		6	15+	80
		9	15+	70
		12	15+	90
Isoniazid	0.05	3	15+	90
		6	15+	100
		9	15+	80
		12	15+	100

TABLE 4

EFFECT OF DELAYED THERAPY WITH SU 3068, SU 3912, AND ISONIAZID ON MOUSE TUBERCULOSIS (H37Rv)

Compound	Concentration in Diet	Delay Before Instituting Treatment	Increase in T50	Per Cent Survivors
	per cent	*days*	*days*	
Su 3068	0.05	0	15+	100
		3	15+	100
		6	15+	100
		9	15+	80
		12	15+	90
		15	15+	70
Su 3912	0.05	0	15+	100
		3	15+	90
		6	15+	60
		9	3.5	30
		12	0	10
		15	0	0
Isoniazid	0.05	0	15+	80
		3	15+	100
		6	15+	80
		9	15+	90
		12	15+	60
		15	15+	100

conducted in order to determine the specific influence of both substances upon the development of lung pathology.

Groups of 20 mice each were infected in the usual manner, and treatment with medicated diets was instituted immediately. Treatment was maintained for a total of twenty-

one days and the experiment was then terminated. Macroscopic involvement of the lungs was evaluated according to the following scheme (7):

0 no visible foci
1+ one or two foci
2+ occasional foci
3+ abundant foci
4+ widespread foci

The results of this experiment are shown in table 5. Although Su 3068 and isoniazid seemed to exert similar activities in mice when the T50 values served as criteria of activity, this experiment clearly established the superiority of isoniazid. A comparison of the numbers of visible lung lesions at comparable dose levels definitely shows that isoniazid exerts a greater suppressive effect on the lesion than Su 3068.

Activity of Su 3068 in guinea pig tuberculosis: As is evident in table 6, this

TABLE 5

INFLUENCE OF VARIOUS DOSES OF SU 3068 AND ISONIAZID ON LUNG INVOLVEMENT IN MOUSE TUBERCULOSIS

Compound	Concentration in Diet	Number of Mice with Gross Lung Involvement of:				
		0	1+	2+	3+	4+
	per cent					
Su 3068	0.20	8	5	4		
	0.10	1	2	10	7	
	0.05			7	11	1
Isoniazid	0.10	8	12			
	0.05	10	10			
	0.01	9	11			
Untreated controls	—				5	15

TABLE 6

ACTION OF SU 3068 AND ISONIAZID ON GUINEA PIG TUBERCULOSIS

Compound	Concentration in Diet	Number of Animals	Average Gross Organ Involvement
	per cent		
Su 3068	0.050	14	19.3
	0.025	14	18.6
	0.010	13	35.4
Isoniazid	0.050	11	12.8
	0.025	14	16.4
	0.010	14	17.0
Untreated controls	—	13	68.1
Pretreatment controls*	—	7	47.1

* The pretreatment controls were sacrificed at the commencement of therapy (twenty-second day post infection) in order to establish the extent of tuberculous involvement.

Note: There was no detectable change in tuberculin reaction in all groups on the thirtieth and sixtieth days of treatment.

substance exerts a significant therapeutic activity at concentrations of 0.05 and 0.025 per cent in the diet. At a concentration of 0.025 per cent, its activity is approximately equal to that of isoniazid at the same concentration. However, at the lower dose of 0.01 per cent, Su 3068 is slightly active; whereas isoniazid still is capable of markedly suppressing the development of lung pathology.

Discussion

These experiments have shown that certain thiazolines and thiazolidinones possess pronounced antituberculous activity in experimentally infected animals. Since the compounds were prepared from thiocarbanilides, it was of interest to compare their activities with those of their parent substances. In all instances, active thiazolines and thiazolidinones were prepared only from active thiocarbanilides; and, with the exception of two compounds, none of these derived substances proved to be more active than their corresponding parent compounds. However, the greater activity of Su 3068 and Su 3912 over that of their precursor, while consistent, may not represent important differences.

Although nothing is known of the metabolic fate of the thiazolines and thiazolidinones, or of the thiocarbanilides, which, as a class, are chemically different, the question naturally arises whether the antituberculous activities of the thiazolines and thiazolidinones differ in mode of action from the activities of the thiocarbanilides from which they were prepared. Cross-resistance tests can readily group together antibacterial substances with either closely related chemical structure or can indicate an identical, if not similar, mechanism of action. An attempt was therefore made by means of this technique to determine whether these three classes of compounds possess a close relationship with respect to mechanism of action upon tubercle bacilli. The attempts of the present writers thus far have afforded no answer to this question, since the development of an appreciable degree of resistance (such as is possible with isoniazid and streptomycin) is complicated by the fact that these substances, at best, exert only fair to moderate *in vitro* activity.

Summary

A number of thiazolines and thiazolidinones have been prepared from corresponding thiocarbanilides and tested for their antituberculous activity. Two of these compounds, namely, 3-(*p*-isoamyloxyphenyl)-2-(*p*-isoamyloxyphenyl-imino)-4-methyl-4-thiazoline·HCl (Su 3068) and 3-(*p*-isoamyloxyphenyl)-2-(*p*-isoamyloxyphenyl-imino)-4-thiazolidinone (Su 3912), were capable of significantly extending the survival rate of infected mice at a concentration as low as 0.01 per cent in the diet. Su 3068, moreover, exerted a marked suppressive effect upon the development of gross pathology in the infected guinea pig.

Sumario

Las Actividades Antituberculosas de las Tiazolinas y las Tiazolidinonas Substituídas

De las tiocarbanilidas correspondientes se han preparado varias tiazolinas y tiazolidinonas, cuya actividad antituberculosa ha sido comprobada. Dos de esos compuestos,

a saber, la 3-(*p*-isoamiloxifenil)-2-(*p*-isoamiloxifenil-imino)-4-metil-4-tiazolina·HCl (Su 3068) y la 3-*p*-isoamilozifenil)-2-(*p*-isoamiloxifenil-imino)-4-tiazolidinona (Su 3912), se mostraron capaces de extender la tasa de sobrevivencia de los ratones infectados a una concentración que no pasaba de 0.01 por ciento en la alimentación. Además, el Su 3068 ejerció notable efecto supresor sobre la formación de patología macroscópica en el cobayo infectado.

Resume

L'activité antituberculeuse des Thiazolines et des Thiazolidinones substituées

Un certain nombre de thiazolines et de thiazolidinones ont été préparées à partir de thiocarbanilides correspondantes et leur activité tuberculostatique a été testée. Deux de ces composés, à savoir; 3-(*p*-isoamyloxyphényl)-2-(*p*-isoamyloxyphényl-imino)-4-méthyl-4-thiazoline·HCl (Su 3068) et 3-(*p*-isoamyloxyphényl)-2-(*p*-isoamyloxyphényl-imino)-4-thiazolidinone (Su 3912) étaient capables d'accroître de façon significative le taux de survie de souris inoculées quand leur concentration dans la ration ne dépassait pas 0,01 pour 100. De plus, Su 3068 exerçait une action inhibitrice sur le développement des lésions macroscopiques chez le cobaye inoculé.

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(3) Konopka, E. A., Eisman, P. C., Mayer, R. L., Parker, F., Jr., and Robbins, S. L.: Antituberculous activity of substituted thioureas: III. Activity in guinea pigs, Am. Rev. Tuberc., 1954, *70*, 130.

(4) Mizzoni, R. H., and Eisman, P. C..: Some thiazolines and thiazolidinones with antituberculous properties, J. Am. Chem. Soc. (in press).

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Notes

THE CHEMICAL CONSTITUENTS OF MYCOBACTERIUM PARATUBERCULOSIS

Although extensive chemical studies have been made on various species of mycobacteria, no report of the constituents of *Mycobacterium paratuberculosis* has been found. A knowledge of the constituents of these bacilli would be of value for improving culture media and diagnostic agents and perhaps would provide a better understanding of the mechanism of infection with *M. paratuberculosis*.

The objects of this experiment were: (*1*) to determine the ash content of a strain of *M. paratuberculosis*, which was grown on synthetic medium, and the percentages of the various minerals of which the ash is composed; (*2*) to determine the nitrogen content of these bacilli and to determine if the nitrogen-containing constituents are extracted by moist heat; (*3*) to determine the quantity of material that can be extracted from *M. paratuberculosis* with petroleum ether anP with alcohol followed by petroleum ether, (*4*) to determine if moist heat will affect the quantity of alcohol-petroleum-ether-soluble material that can be extracted from the bacilli; and (*5*) to determine the saponification number of the alcohol-petroleum-ether extract.

All bacilli used in these studies were grown on Dorset and Henley's synthetic liquid medium. After an incubation period of nine weeks at 38° C., the cultures were divided into three lots. The cultures in *Lot 1* were filtered to separate the organisms from the culture fluid, and the organisms were then washed three times with distilled water to remove medium constituents. *Lots 2* and *3* were treated the same as *Lot 1* except that *Lot 2* was steamed for three hours in an Arnold sterilizer and *Lot 3* was autoclaved for three hours at 15 pounds of pressure prior to separation. A portion of the washed organisms from *Lot 3* was further autoclaved for forty-eight hours at 15 pounds of pressure, and another portion from the same lot was autoclaved for one hundred and twelve hours at 15 pounds of pressure.

The organisms from each lot were dried at 105° C. for sixteen hours, and the culture filtrates from the corresponding lots were made into johnins.

Standard analytical procedures of the Association of Official Agricultural Chemists[1] were used for obtaining ash and in determining the saponification number and quantities of calcium, phosphorous, and magnesium. Spectrophotometric methods were used to determine iron, potassium, sodium, and sulfur contents.[2] All analyses were made in triplicate and the results were averaged.

The ash content was obtained, and its principal constituents were determined quantitatively. The petroleum-ether-soluble portion was obtained by extraction for sixteen hours in a Soxhlet apparatus. The alcohol-petroleum-ether-soluble portion was obtained by first extracting the dried bacilli with hot absolute alcohol for four hours in a Soxhlet apparatus and then extracting them with hot petroleum ether for 16 hours in the same apparatus. The combined alcohol-petroleum-ether-extractible material was evaporated to dryness, weighed, and calculated as the percentage of total dry weight of the bacilli.

Nitrogen determinations were made on the three lots of organisms after the alcohol-petroleum-ether-soluble portion had been removed. Similar determinations were made on johnins prepared from the three lots of culture filtrates.

[1] Official Methods of Analysis of the Association of Official Agricultural Chemists, ed. 7, Association of Official Agricultural Chemists, Washington 4, D. C., 1950.

[2] Through the courtesy of Drs. L. E. Ensminger, T. B. Hagler, and R. D. Rouse, of the Alabama Agricultural Experiment Station.

TABLE 1

MINERAL CONSTITUENTS OF M. PARATUBERCULOSIS COMPARED WITH THOSE OF M. TUBERCULOSIS

	M. Paratuberculosis	*M. Tuberculosis**
Dried Organisms		
	per cent	
Ash	2.69	8.05
Ash Constituents		
	per cent	
Calcium	2.20	0.00
Iron	0.72	0.56
Magnesium	13.40	3.99
Phosphorous	21.00 (64.3 PO_4)	28.63
Potassium	7.40	12.78
Sodium	3.10	1.86
Sulfur	9.30	2.34

* Strain H37, Wells and Long[3]

The quantitative analysis of the ash of unheated *M. paratuberculosis* compared with a similar analysis of *M. tuberculosis* grown on Long's synthetic medium for seven weeks[3] is shown in table 1. The saponification number, lipid and nitrogen contents of *M. paratuberculosis*, and the nitrogen content of johnins together with the results obtained by Renfrew,[4] and Long and Campbell[5] are shown in table 2. The procedure used by Long and Campbell consisted of first extracting the bacilli with hot absolute alcohol, then extracting the residue of the alcohol extraction with petroleum ether and adding this to the petroleum-ether extract obtained from the bacilli.

The percentage of phosphorous and potassium in the ash of *M. paratuberculosis* was less than in the ash of *M. tuberculosis*; however, the calcium, magnesium, sulfur, and sodium contents of *M. paratuberculosis* were higher than those reported for *M. tuberculosis* by Wells and Long,[3] who stated that changing the concentration of the elements in the medium may alter the quantity of each element in the ash. This may also be true for *M. paratuberculosis*.

Paratuberculosis bacilli, like other mycobacteria, contain a relatively large amount of lipid material. The marked increase in the quantity of alcohol-petroleum-ether-extractible material after prolonged heating substantiates reports of others[6, 7] who found that much of the lipid content is firmly bound in the cells and cannot be extracted by normal procedures. The lipid content of the unheated bacilli is quite similar to that reported by Long and Campbell,[5] and by Anderson,[6] in the human and bovine strains of tubercle bacilli and in *Mycobacterium phlei*; however, this is approximately twice the lipid content

[3] WELLS, H. H., AND LONG, E. R.: The Chemistry of Tuberculosis, Williams & Wilkins Company, Baltimore, 1932.

[4] RENFREW, A. G.: J. Biol. Chem., 1929, *83*, 569.

[5] LONG, E. R., AND CAMPBELL, L. K.: Am. Rev. Tubere., 1922, *6*, 636.

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[7] ARONSON, H.: Berl. klin. Wchnschr., 1898, *35*, 484.

TABLE 2

SAPONIFICATION NUMBER, LIPID AND NITROGEN CONTENTS OF M. PARATUBERCULOSIS AND NITROGEN CONTENT OF JOHNINS

Species	Saponification Number	Lipid Content (Per Cent)		Bacillary Nitrogen	Johnin Nitrogen
		Petroleum-Ether Extract	Alcohol-Petroleum-Ether Extract		
				*per cent**	*mg./ml.*
M. paratuberculosis (unheated)	152	16.71	22.47	8.54	1.70
M. paratuberculosis (steamed 3 hours)	—	18.58	21.70	9.07	2.35
M. paratuberculosis (autoclaved 3 hours)	—	18.91	23.67	9.05	2.70
M. paratuberculosis (autoclaved 48 hours)	—	—	41.40	—	—
M. paratuberculosis (autoclaved 112 hours)	—	—	44.75	—	—
M. tuberculosis, strain H37 (unheated)	131†	—	22.70†	10.72‡	—
M. tuberculosis var. *bovis* (unheated)	139†	—	22.30†	—	—
M. tuberculosis var. *avium* (unheated)	194†	—	11.00†	9.33‡	—
M. leprae (unheated)	188†	—	9.70†	—	—
M. phlei (unheated)	—	—	20.20†	—	—

* Nitrogen determined on alcohol-petroleum-ether extracted organisms

† From Long and Campbell[5]

‡ From Renfrew[4]

of the avian type of tubercle bacilli and leprosy bacilli. The saponification number of *M. paratuberculosis* was between that of the lipids of the human and bovine tubercle bacilli on the one hand and those of avian tubercle bacilli and leprosy bacilli on the other.

When the percentage of nitrogen present is multiplied by the protein to nitrogen ratio of 6.25:1, it becomes evident that the bacilli are composed of at least 50 per cent protein after the alcohol-petroleum-ether-soluble portion has been removed. The nitrogen content is of particular interest in this investigation because the material responsible for johnin reactions in infected animals is primarily nitrogenous. Heating the cultures for three hours, as in the preparation of standard johnin, resulted in an increase of nitrogen in this johnin as compared with johnin prepared from unheated cultures. However, the non-nitrogenous material was extracted faster from the bacilli than the nitrogenous material since the unheated bacilli had a slightly higher nitrogen content on a percentage basis than the heated bacilli.

In summary: Chemical determinations were made on *Mycobacterium paratuberculosis*. The dry bacilli had an ash content of 2.69 per cent. The principal constituents of the ash were: calcium, 2.2 per cent; iron, 0.72 per cent; magnesium, 13.4 per cent; phosphorous, 21.0 per cent; potassium, 7.4 per cent; sodium, 3.1 per cent; and sulfur, 9.3 per cent. The alcohol-petroleum-ether-extractible portion was 22.47 per cent of the dry unheated bacilli. This extractible portion was found to increase after prolonged autoclaving. For example, after heating the organisms for one hundred and twelve hours

at 15 pounds of steam pressure, 44.75 per cent of their weight was alcohol-petroleum-ether-extractible.

The nitrogen content was found to be 8.54 per cent of the weight of the alcohol-petroleum-ether-extracted bacilli.

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SPUTUM EXAMINATION FOLLOWING BRONCHOSCOPY

Its Significance in Tuberculosis

The diagnostic value of secretions obtained by aspiration at the time of bronchoscopy has long been recognized. More recently, attention has been directed to the sputum produced following the procedure[1, 2] or during topical anesthetization[3] as a similarly fruitful source of diagnostically significant results. There is little doubt that the mechanical irritation of topical anesthetization and bronchoscopy causes a transiently increased production of sputum, collection of which is facilitated by an unusually effective cough. These circumstances appear to offer additional means of establishing the bacteriologic status of the tuberculous patient who is under treatment with antituberculous drugs. The present study was undertaken to assess the possible advantages of routine collection and examination of the post-bronchoscopy sputum in such patients and particularly to compare the results thus obtained with those obtained by bronchoscopic aspiration.

Beginning in March, 1956, sputum was routinely collected for a 48- to 72-hour period following each bronchoscopy and was submitted for bacteriologic study, along with the usual bronchoscopic aspirate. At least one microscopic examination and one culture of each specimen were performed. In addition, the bacteriologic status of every patient was studied by sputum microscopy at monthly intervals and by less frequent sputum and gastric cultures. During the 12-month period ending in February, 1957, 215 bronchoscopic examinations were performed on 192 patients. After excluding all instances of unproved pulmonary tuberculosis, therapeutic bronchoscopy, and incomplete bacteriologic data, there remained as suitable candidates for study 143 patients who underwent 165 bronchoscopies. All patients were receiving antituberculous drug therapy at the time of bronchoscopy. The bacteriologic course of each individual was charted, including the results of bronchoscopic aspiration and post-bronchoscopy sputum collection. The data thus obtained form the basis of this report.

As mentioned, 143 patients and 165 bronchoscopies were susceptible of analysis. In 81 patients (57 per cent) who underwent 89 bronchoscopic examinations, both the bronchial aspirate and the post-bronchoscopy sputum were negative for tubercle bacilli on microscopy or culture. The findings in the remaining 62 patients (43 per cent), who underwent 76 bronchoscopies, were positive for tubercle bacilli at least once by microscopy or culture of the bronchial aspirate or post-bronchoscopy collection. However, since 11 of these 76 bronchoscopies yielded negative results on both bronchial aspirate and post-bronchoscopy collection, the absolute number of bronchoscopies with positive results was 65, or 39 per cent of the total number of examinations (table 1).

Of the completely "negative" group, 45 were followed bacteriologically as inpatients for periods ranging from 3 to 19 months, with an average duration of 5.8 months. Sputum smears were obtained routinely at monthly and often at fortnightly intervals, and bacteriologic negativity was confirmed by frequent sputum or gastric cultures or both. For purposes of this study, bacteriologic data were not recorded following medically approved discharge, self-release, or definitive surgery. During this period subsequent to bronchoscopy, only 5 of the 45 patients in the "negative" group yielded specimens positive for tubercle bacilli. Two patients had sputum positive on microscopy within one month of bronchoscopy and negative examinations thereafter. One patient had sputum positive for acid-fast bacilli on microscopy two months after bronchoscopy, and one patient had similarly positive findings four months afterward. A fifth patient

[1] Fourestier, M.: Poumon, 1955, *11*, 668.
[2] Wyborney, V. J.: Am. Rev. Tubere., 1957, *75*, 854.
[3] Lowenstein, B., and Gale, D.: Am. Rev. Tuberc., 1956, *74*, 977.

TABLE 1

EXAMINATION FOR TUBERCLE BACILLI IN BRONCHOSCOPIC ASPIRATION AND POST-BRONCHOSCOPY SPUTUM COLLECTION

	Patients	Bronchoscopies	
Negative by microscopy and culture of bronchial aspirate and of post-bronchoscopy sputum	81	89	
Positive on at least one examination by microscopy and/or culture of bronchial aspirate and/or post-bronchoscopy collection	62	76	
Aspirate and collection both positive			26
Aspirate positive, collection negative			15
Aspirate negative, collection positive			24
Both negative			11
	143	165	

had sputum negative for tubercle bacilli for nine months after bronchoscopy except for one positive culture during the fourth month and one positive smear during the sixth month.

The second group comprised 62 patients who had positive findings on at least one occasion, either by microscopy or culture of the bronchial aspirate or of the post-bronchoscopy collection. As previously noted, 11 of the 76 bronchoscopies performed on these patients yielded completely negative bacteriologic data. In 26 of the remaining 65 bronchoscopies, tubercle bacilli were demonstrated by microscopy or culture, or by both procedures, in both the bronchial aspirate and the post-bronchoscopy sputum. In 15 other patients the aspirate was positive and the post-bronchoscopy collection negative and in an additional 24 the reverse was true. Thus, in this group, the post-bronchoscopy collection was solely responsible for the demonstration of tubercle bacilli in 24 instances and the bronchial aspirate in 15 instances.

Analysis of the over-all distribution of the positive results by the bacteriologic method employed showed that the bronchial aspirate was positive for tubercle bacilli on 30 occasions by microscopy and a like number of times by culture. The results of the microscopic examinations did not always coincide with those of the cultures. Of the post-bronchoscopy sputum collections that were positive for tubercle bacilli, the demonstration was made in 41 by microscopy and in 33 by culture. Again, the results obtained by smear did not necessarily agree uniformly with those of the cultures.

There were 28 examinations in which only one method of specimen collection and only one bacteriologic technique afforded a positive result. Thus, direct smear of the bronchial aspirate yielded the only positive result in 5 instances, and culture of the bronchial aspirate the only positive result in 7. On 11 occasions a direct smear of the post-bronchoscopy sputum afforded the only positive result, and on 5 occasions culture of this material yielded the only positive result.

Thirty-one patients in the "positive" category had had secretions negative for tubercle bacilli by microscopy and culture, or gastric culture, for at least one month prior to bronchoscopy. In this group, periods of antecedent noninfectious status ranging from 2 to 49 months had been recorded, with an average of 8 months. The average period of absence of tubercle bacilli from sputum or gastric washings prior to bronchoscopy was 8.4 months in those patients who were positive by both bronchial aspirate and post-bronchoscopy collection, 7.8 months in those positive by bronchial aspirate alone, and 7.9 months in those positive by post-bronchoscopy collection alone. These differences do not appear significant.

The advent of effective antituberculous chemotherapy has rendered it more difficult to ascertain the true bacteriologic status of many patients. In addition to the usually early disappearance of tubercle bacilli from the sputum, there often results a marked diminution in such associated symptoms as cough, thus rendering it more difficult to obtain representative secretions for examination. Later, prompt recognition of the emergence of resistant strains by the usual methods of smear examination may be delayed for similar reasons.

On the basis of the data submitted, it is believed that routine collection and examination of the sputum produced following bronchoscopic instrumentation constitutes a practical and useful adjunct to the more customary methods of determining bacteriologic status. Of especial significance is the fact that the post-bronchoscopy examination demonstrated the presence of tubercle bacilli on 24 occasions when the bronchial aspirate was negative. This assumes further importance when it is noted that the post-bronchoscopy collection failed to be positive for tubercle bacilli on only 15 occasions when the concomitant bronchial aspirate was positive. It would thus appear that the post-bronchoscopy collection is a somewhat more sensitive indicator of bacteriologic status than is the bronchial aspirate.

The prognostic value of the post-bronchoscopy collection should also be considered, since only 5 of 45 patients who had negative sputum findings by microscopy and culture of both the bronchial aspirate and post-bronchoscopy collection subsequently had sputum positive for tubercle bacilli in a period of follow-up which averaged 5.8 months. The results of Fourestier[1] are equally impressive. In his series, only 16 per cent of patients whose bronchial aspirate and post-bronchoscopy collection were both negative for tubercle bacilli were subsequently shown to be discharging tubercle bacilli in the course of one year. Thus, when the absence of tubercle bacilli from both the bronchial aspirate and post-bronchoscopy collection has been established, there is a high degree of probability that the sputum will remain negative for tubercle bacilli for a considerable period subsequently. Of practical importance under such circumstances would be the added assurance that resection might safely be undertaken without additional antimicrobial therapy.

In summary: In a series of patients with proved pulmonary tuberculosis who underwent bronchoscopy, the bacteriologic results obtained by bronchoscopic aspiration were compared with those secured by examination of the sputum collected during the 48- to 72-hour period following bronchoscopy. In addition, these data were studied in relation to the long-term bacteriologic status of each patient. All patients were under treatment with appropriate antituberculous drugs at the time of bronchoscopy. It was found that the sputum produced following bronchoscopy was a more fruitful source of positive bacteriologic data than was the secretion aspirated at the time of bronchoscopy. Bacteriologic negativity of both the bronchoscopic aspirate and post-bronchoscopy sputum proved of considerable prognostic import since such patients rarely became infectious during the period of subsequent observation in the hospital. It is believed that the routine collection of sputum for a 48-to 72-hour period following bronchoscopy is a practical diagnostic adjunct which often affords valuable bacteriologic data.

THORACIC SURGERY SERVICE, SANATORIUM DIVISION
BOSTON CITY HOSPITAL
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November 13, 1957

ANTITUBERCULOUS VACCINATION IN THE GUINEA PIG WITH NON-LIVING VACCINES[1]

In 1902, Pearson and Gilliland,[2] writing on the topic of antituberculous vaccination, expressed the opinion that "the whole story of immunization has led investigators to suspect a solution to tuberculosis immunity through the use of a living virus." This view has changed very little in the ensuing fifty years and, as Gaisford has pointed out recently, "one has come to think in terms of BCG (*living*) when talking of protective vaccination against tuberculosis."[3] An analysis of the experiments with non-living vaccines against tuberculosis, which have been published over the past seventy years, does not support this view. The present writer has recently made an extensive review of the literature,[4] to be published shortly,[5] and cannot escape the conclusion that non-living vaccines have consistently been shown to be as efficacious as living ones when the special experimental circumstances which govern their use have been considered in the design of the experiment. The non-living vaccines which have been found to be efficacious include intact tubercle bacilli killed by a variety of physical and chemical methods, and a number of bacillary fractions. Such preparations have been shown to be as immunogenic as living BCG and vole bacillus in a variety of laboratory and domestic animals; equally good results have been obtained in several limited human trials. Indeed, a great majority of all of the experimental reports on the comparative immunogenicity of living and non-living vaccines against tuberculosis have shown the non-living vaccines to be at least equally protective. It seems paradoxical, therefore, that the superiority of living vaccines has been maintained so widely, with an intractability that already prompted Langer[6] in 1927 to describe the opinion as "ein unbeweisbares" dogma. An attempt to explain this paradox will be made more fully elsewhere, but can be outlined briefly here.

In the period immediately before and after the discovery of the tubercle bacillus, a number of effective living vaccines, consisting of attentuated organisms, were discovered against a variety of infectious diseases, e.g. anthrax (1881)[7], swine erysipelas (1882),[8] rabies (1885),[9] cholera (1892),[10] and contagious abortion of cattle (1906),[11] to name only a few. It also appeared that attenuation to the point at which the microorganisms could no longer reproduce themselves in tissues or induce histopathologic changes reduced or

[1] The experiments described in this communication were carried out under the tenure of a Merck Senior Postdoctoral Fellowship, and were supported in part by the Medical Research Council of Great Britain.

[2] Pearson, L., and Gilliland, S. R.: Philadelphia M. J., 1902, *10*, 842.

[3] Gaisford, G.: Brit. M. J., 1955, *2*, 1101.

[4a] Weiss, D. W.: The Induction of Protective Immunity in Mice and Guinea Pigs with Non-Living Vaccines. Thesis submitted to the Faculty of Medicine, University of Oxford, October, 1957.

[5] Dubos, R. J., *et al.*: J. Exper. Med., 1953, *97*, 221.

[6] Jespersen, A., and Magnusson, M.: Acta path. *et* microbiol. Scandinav., 1956, *39*, 431.

[5] Weiss, D. W.: In preparation for submission to Am. Rev. Tubere.

[6] Langer, H.: Deutsche med. Wchnschr., 1927, *53*, 597.

[7] Pasteur, L.: Compt. rend. Acad. d. sc., 1881, *92*, 209.

[8] Pasteur, L., Compt. rend. Acad. d. sc., 1882, *95*, 1120.

[9] Pasteur, L.: Compt. rend. Acad. d. sc., 1885, *101*, 765.

[10] Haffkine, W. M.: Compt. rend. Soc. de biol., 1892, *4*, 635, 671.

[11] Bang, B.: J. Comp. Path. & Therap., 1906, *19*, 191.

abolished their vaccinating powers. The view arose, therefore, that in order to be effective a vaccine must be viable and still somewhat "virulent." This, together with the common clinical observation that survivors of certain diseases are endowed with an increased resistance to further episodes, gave rise to the theory that only disease can protect against *further* disease.

This hypothesis was strengthened in the case of tuberculosis by two other circumstances. Commencing with Koch's work on killed tubercle bacilli and tuberculins,[12] trials with non-living materials derived from tubercle bacilli were directed for decades towards two distinct goals, therapy and prophylaxis.[13] Successive therapeutic failures with such materials also caused them to fall into disrepute as prophylactic agents. Second, a number of early investigators failed to produce immunity with non-living vaccines, or were able to do so only with the simultaneous evocation of toxic symptoms.[14]

The efficacy of non-living vaccines against infectious diseases other than tuberculosis has since been recognized, but the early conviction of their uselessness against tuberculosis has been kept alive by the repeated failure of some investigators to elicit protective immunity by their use. Some of the reasons for these failures have been discussed recently[15] and arise largely from the fact that the comparisons of living and non-living vaccines are usually made in terms of the former. The same dose and route of administration are usually employed and, when the dose of non-living vaccine *is* varied, it is usually in terms of two to one hundred times the quantity of living vaccine. This approach ignores the fact that only a viable vaccine can multiply or synthesize effective antigen *in vivo*. While some degree of immunity will be produced even by very small quantities of living vaccine, fifty or even one hundred times that amount of a non-living preparation may still be too little. When the vaccine is injected intradermally, further loss may result by mechanical expulsion from a suppurating vaccinal lesion. On the other hand, an injection of too large a quantity of a killed vaccine induces toxic reactions which can vitiate the immunogenic ones. There are still other experimental circumstances which affect the activity of non-living vaccines; a prominent one is the method of killing.

It is unfortunate that non-living vaccines continue to be condemned on the basis of artificial experimental failure, because they offer a number of categorical advantages over living vaccines. They can be completely standardized and stored for long periods of time, are entirely safe, and, perhaps most important, they offer the possibility of separating the immunogenic from the tuberculin-sensitizing antigens of the tubercle bacillus and the consequent development of a non-sensitizing vaccine.

A reinvestigation into the immunogenicity of non-living vaccines was begun some years ago in the laboratories of the Rockefeller Institute for Medical Research.[16] The results obtained in mice, which are already published,[17-19] again indicated the efficacy of such vaccines. Further experiments, using the guinea pig, were carried on in the laboratories of

[12] Koch, R.: Deutsche med. Wchnschr., 1897, *23*, 209.

[13a] Dreyer, G.: Lancet, 1923, *2*, 40.

[b] Editorial: Lancet, 1935, *2*, 780, 892.

[14a] Trudeau, E.: Trans. A. Am. Physcns., 1894, *9*, 168.

[b] Smith, T.: J.A.M.A., 1917, *68*, 764.

[15] Weiss, D. W., and Wells, A. Q.: Tubercle, 1956, *37*, 137.

[16] Dubos, R. J., *et al.*: J. Exper. Med., 1953, *97*, 221.

[17] Weiss, D. W., and Dubos, R. J.: J. Exper. Med., 1955, *101*, 313.

[18] Weiss, D. W., and Dubos, R. J.: J. Exper. Med., 1956, *103*, 73.

[19] Dubos, R. J., Weiss, D. W., and Schaedler, R. W.: Am. Rev. Tuberc., 1956, *75*, 781.

TABLE 1

EFFECT OF DOSE AND ROUTE OF ADMINISTRATION ON IMMUNOGENICITY OF PHENOL-KILLED TUBERCLE BACILLI

Vaccine	Dose	Route	Mean Survival Time after Infection	Per Cent Increase Over Saline Controls	Significance Level Over Saline Controls	Change in Weight Per Animal on Seventh Post-Vaccination Day Over Initial Weight
	mg.		*days*		(*P*)	*gm.*
Phenolized tubercle bacilli..	1.5	I.P.*	123	59	<0.001	+60
Phenolized tubercle bacilli..	3.0	I.P.	105	36	<0.001	+40
Phenolized tubercle bacilli..	6.0	I.P.	116	50	<0.001	+35
Phenolized tubercle bacilli..	12.0	I.P.	115	49	<0.001	+ 5
Phenolized tubercle bacilli..	24.0	I.P.	90	16	0.070	−20‡
Phenolized tubercle bacilli..	3.0	S.C.†	90	16	0.100	+50
Phenolized tubercle bacilli..	6.0	S.C.	111	44	<0.001	+50
Phenolized tubercle bacilli..	12.0	S.C.	85	10	0.380	+50
Phenolized *M. phlei*.....	1.5	I.P.	80	4	0.800	+40
Living BCG, 0.5 ml.....		S.C.	119	54	<0.001	+55
Saline, 0.5 ml.....		I.P.	77	—	—	+60

* I.P.: Injected intraperitoneally.

† S.C.: Injected subcutaneously.

‡ One animal died after vaccination.

the Sir Wm. Dunn School of Pathology, Oxford, and the Microbiological Research Establishment of the Ministry of Supply at Porton, England. The results obtained in more than 1,500 guinea pigs will be published in the near future.[20] A few of the findings are presented here to indicate that non-living vaccines can consistently induce as high a degree of protective immunity in the guinea pig, without inducing frank toxicity, as does living BCG and that a non-sensitizing vaccine may be developed.

Groups of 15 young male guinea pigs (weighing between 300 and 400 gm.) were vaccinated either intraperitoneally or subcutaneously with quantities of 1.5 to 24.0 mg. of phenol-killed, acetone-washed tubercle bacilli suspended in saline. The bacilli were of the Pasteur BCG strain and were prepared according to a previously published method.[17] One group of control animals was given saline alone, intraperitoneally; another, 0.5 ml. of a suspension of living BCG, subcutaneously, containing between 2 and 20 million living bacilli; and a third, 1.5 mg. phenol-killed, acetone-washed *M. phlei*, intraperitoneally, to ascertain whether this tubercle bacillus-like organism could induce a degree of "nonspecific" immunity.

The vaccinal lesions induced by the subcutaneous injection of killed bacilli were found to be smaller than those arising from the injection of living BCG.

Six weeks after vaccination, the animals were infected by inhalation of a cloud of virulent tubercle bacilli of the Vallée strain, with an estimated lung retention of 6,000 bacilli per animal. The technique for inhalation-infection has been described previously.[21]

The following data are presented in table 1: mean survival time after infection; per cent increase in mean survival time over the saline controls; statistical significance of this comparison; and gain or loss in weight per animal, based on the weights of a sample group of 5 animals from each experimental group on the seventh post-vaccination day. Good correlation was found between the weight curves and general appearance of the animals; re-

[20] WEISS, D. W., *et al.*: In preparation for submission to Am. Rev. Tuberc.

[21] HENDERSON, D. W.: J. Hyg., 1952, *50*, 53.

TABLE 2

IMMUNOGENICITY AND ALLERGENICITY OF METHANOL EXTRACT

Vaccine	Diameter of Induration (Average of 8 Animals) at Site of Intradermal Injection of 200 TU of PPD after 24 Hours *mm.*	Mean Survival Time *days*	Per Cent Increase Over Saline Controls
Vaccination-Challenge Interval: 8 Weeks			
Methanol extract, 3.0 mg., I.P.*	2‡	100	26
Methanol extract, 6.0 mg., I.P.	2	79	0
Living BCG, 0.5 ml., S.C.†	19	125	58
Saline, 0.5 ml., I.P.	2	79	—
Vaccination-Challenge Interval: 26 weeks			
Methanol extract, 3.0 mg., I.P.	0	103	20
Methanol extract, 6.0 mg., I.P.	3	94	9
Living BCG, 0.5 ml., S.C.	18	126	47
Saline, 0.5 ml., I.P.	2	86	—

* I.P.: Injected intraperitoneally.

† S.C.: Injected subcutaneously.

‡ A diameter of induration less than 5 mm. is not considered to be a positive reaction.

tardation of weight gain due to vaccination was always found to be greatest on the seventh day.

All of the animals died after experimental infection and a postmortem examination revealed advanced tuberculosis in every instance. Vaccination succeeded, however, in prolonging the survival time, and several of the groups vaccinated with non-living vaccine were as protected as the animals vaccinated with living BCG. Small quantities of killed vaccine appeared to be as efficacious as, or superior to, the larger quantities employed. The most effective doses of killed vaccine caused no appreciable retardation in weight gain. *M. phlei* gave no protection.

Similar results were obtained in other experiments in which the challenge was by intraperitoneal or intramuscular injection. Repeated injections or emulsification in Freund's adjuvant enhanced the immunogenicity of the killed bacilli and reduced the quantity necessary to obtain maximal protection. The optimal dose was usually small and was readily exceeded. Toxic manifestations were not seen, until several times the optimal dose was given. The animals were found to be as immune when challenged six months after vaccination as they had been after six weeks. Autoclaving the phenol-killed bacilli somewhat reduced their immunogenicity.

Vaccination with intact, phenolized bacilli rendered the animals tuberculin sensitive. In contrast, an extract of the killed bacilli soluble in hot methyl alcohol was found to be capable of inducing a degree of protective immunity without tuberculin hypersensitivity. This extract was prepared according to a previously published method,[18] and appears to be similar in some respect to the "antigène méthylique" described by Nègre and Boquet.[22]

Young male guinea pigs in groups of 16 were vaccinated intraperitoneally with 1, 3, or 6

[22] NÈGRE, L.: Les Lipoides Dans les Bacilles Tuberculeux et la Tuberculose, Masson et Cie, Paris, 1950.

TABLE 3

IMMUNOGENICITY OF METHANOL EXTRACT—EXTRACTION RESIDUE COMBINATION

Vaccine	Diameter of Induration (Average of 8 Animals) at Site of Intradermal Injection of 2,000 TU of PPD after 24 Hours	Median‡ Survival Time	Per Cent Increase over Saline Controls
	mm.	*days*	
Methanol extract, 1.0 mg., I.P.*	0	81	0
Extraction residue, 0.3 mg., I.P.	7	94	16
Methanol extract, 1.0 mg. + extraction residue, 0.3 mg., I.P.	7	126	56
Living BCG, 0.5 ml., S.C.†	20	134	65
Saline, 0.5 ml., I.P.	0	81	—

* I.P.: Injected intraperitoneally.

† S.C.: Injected subcutaneously.

‡ Median instead of mean survival time is given here because several animals were still alive in this experiment at the time of writing. In all other experiments the mean and median values were found to be in close agreement.

mg. of methanol extract suspended in distilled water; control groups were vaccinated intraperitoneally with water or subcutaneously with living BCG. The animals were tuberculin tested fifty-four days after vaccination by the intradermal injection of 200 tuberculin units (TU) of PPD. Two days later, half the animals in each group were challenged by the intramuscular injection of a diluted culture of highly virulent bovine tubercle bacilli ("Branch" strain). The other animals were similarly challenged twenty-six weeks after vaccination. The tuberculin test and survival data are shown in table 2.

It may be seen that the extract induced a degree of protective immunity only half as good as that given by living BCG, but this immunity was not accompanied by skin hypersensitivity to PPD. Similar results were obtained in repeated experiments. It was also found that Freund's adjuvant enhanced the efficacy of the extract, but with the doses employed (1.0 to 12.0 mg.) the immunity was never as great as that given by live vaccine. No toxic symptoms were elicited by the extract. In contrast to the reports of Nègre that the immunity bestowed on guinea pigs by their antigène méthylique had a duration of only three to four months,[22] the protection bestowed by the methanol extract appeared to be undiminished after six months.

It has been suggested previously that some components of the tubercle bacillus may act as adjuvants for other, specifically immunogenic moieties.[17] To explore this possibility, experiments were designed to test the immunizing activity of combinations of methanol extract and extraction residue.

Groups of 20 young guinea pigs (10 of each sex per group) were vaccinated intraperitoneally with one of the following substances in saline: 1.0 mg. of methanol extract (a subeffective amount); 0.3 mg. of extraction residue; a combination of the extract and residue; living BCG, subcutaneously; and saline, intraperitoneally. Seven weeks later, the animals were tuberculin tested with 2,000 TU of PPD. They were challenged fifteen weeks after vaccination by the intramuscular injection of a diluted culture of Vallée strain. The tuberculin test and survival data are presented in table 3.

In summary: The results show that in an experiment in which neither methanol extract

[22] NÈGRE, L.: Ann. Inst. Pasteur, 1952, *83*, 429.

nor extraction residue alone was able to induce protective immunity, the combination was as efficacious as living BCG. The extraction residue induced a slight degree of tuberculin hypersensitivity, although considerably less than the living BCG or, as revealed in other experiments, equivalent amounts of intact phenolized bacilli. Experiments are in progress to determine whether physical treatment of the extraction residue can destroy completely its sensitizing powers without reducing its immunizing activity. Preliminary results suggest that this may be possible.

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November 27, 1957

[24] The writer wishes to express his gratitude to Dr. D. W. Henderson for making available to him the excellent facilities of the Microbiological Research Establishment Laboratories at Porton, England.

Books

DAS SOGENANNTE ALVEOLARZELLENKARZINOM. By Hermann Eck. Pp. 71, Veb Georg Thieme, Leipzig, 1957. Price, 14 German marks (East).

Alveolar cell carcinoma, synonymous with pulmonary adenomatosis, has aroused interest among pathologists with respect to its origin and classification ever since it was first described by the French physician Malassez in 1876. At present, the view is widely held that alveolar carcinoma has a multicentric origin from respiratory epithelium rather than primary tumor site. It is supposedly different from the ordinary bronchial carcinoma with respect to frequency of occurrence, sex distribution, and pattern of metastatic spread. The histologic picture of this relatively rare tumor shows well-differentiated cylindrical cells not penetrating into or destroying the alveolar membrane.

Dr. Eck, the author of the present study, has in the past eight years contributed several papers on this subject. He holds the not undisputed view that the features supposedly differentiating pulmonary adenomatosis from bronchial carcinoma are in reality not existent. The monograph under review comprises the available evidence in support of his view.

Dr. Eck first takes issue with the impression that incidence, age, and sex distribution, pattern of metastatic spread, and the time elapsing until manifestation of clinical symptoms clearly differentiate the alveolar from the bronchial carcinoma. In reviewing the literature (which includes also more recent American publications) he shows that with an accumulation of larger numbers of reports true differences between these two tumors disappear. He then elaborates upon the question of whether a primary tumor is really absent or only so small and insignificant in size that it is not detectable with standard autopsy techniques. In several well-chosen examples from his own autopsy material, which were previously followed clinically with great accuracy, he demonstrates the possibility that typical pulmonary adenomatosis can arise from bronchial carcinoma or even extrapulmonary tumors. The typical form of growth represents only a relatively rare pattern of spread, which need not necessarily occur in the lungs but can also be seen in the liver. While the possibility of an alveolar carcinoma cannot be excluded, it is probable that all cases described so far as such may have originated from the bronchial epithelium or—more rarely—from extrapulmonary tumors.

A good number of well-printed pictures illustrate the author's arguments which unfortunately are sometimes brought forward in a strongly polemic style which might make understanding difficult for the English-speaking reader not thoroughly familiar with the German language. However, the importance of this study justifies recommending it to all investigators interested in this special aspect of cancer research.

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SOCIAL-MEDICAL INVESTIGATIONS ON TUBERCULOSIS IN THE COUNTY OF HORDALAND. By K. Engedal. Pp. 126, A. S. John Griegs Boktrykkeri, Bergen, 1956.

The author presents the results of an extensive research study of the medical and social aspects of tuberculosis in the County of Hordaland in Norway. The medical information on the tuberculosis problem in this well-defined geographic area is carefully correlated with social and economic studies in this population group. The author has made an

ambitious effort to develop a better understanding of the way in which tuberculous disease affects the individual in the framework of his family and his community.

The methods of gathering data to document the medical and socioeconomic status of the patients are described in detail. The actual inquiry forms which were used in the research studies and innumerable statistical tables are incorporated in this monograph.

This book contains much information pertinent to the interests of the sociologist and public health worker. Unfortunately, this large collection of data necessitates a degree of effort to the reader which sharply limits the value of the monograph to the physician.

K. Deuschle
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Electrocardiography. Fundamentals and Clinical Application, ed. 2. By Louis Wolff. Pp. 342, W. B. Saunders Company, Philadelphia, 1956. Price, $7.00.

In his preface to the first edition, published seven years ago, Dr. Wolff encourages the student electrocardiographer to develop not only skill in interpretation of records but also a somewhat nebulous quality which he speaks of as "restraint."

"Restraint" and a reliance upon known and well-charted principles are the fundamentals which Dr. Wolff brings to the exploration of the electrocardiogram. Extraneous, complicated, and controversial materials are minimized whenever clarity demands, even at the expense of completeness in some aspects. The approach is never one of patterns or mnemonics; the neophyte is constantly counseled by both rule and example to recall and to exercise basic principles of physiology involved in electrical phenomena observed.

To emphasize this the book is divided into two parts. The first part is devoted exclusively to an examination of fundamentals and includes discussion of electric field forces both as physical and biologic phenomena. The reader is introduced to the basic unit—the dipole—as a generator of forces, and to the volume conductor and its divisions as the only practical medium available for measure of these forces. The relation of one to the other is clearly demonstrated with reference to both measured and theoretical effects of isolated muscle strips and dipoles.

The second section, entitled "Clinical Electrocardiography," is introduced with a brief discussion of the instruments used and the nomenclature classically employed, and proceeds with a detailed discussion complete with statistical tables of the normal electrocardiogram. Attention is then directed to the pathologic states encountered in electrocardiography; and the manner in which basic physiology and, hence, the electrocardiogram, is altered from normal is discussed in detail. Each of these latter discussions is enhanced through the broad use of tabulated material and illustrations from the author's files, making this section particularly a useful reference for the experienced electrocardiographer as well as an introduction for the beginning student and practitioner.

In the second edition, Dr. Wolff, in addition to increasing the number of illustrations and examples and expanding the tables with more current material, introduces two major additions to the original text.

The first of these is a section devoted to arrhythmias which he treats in a manner similar to that used in the first two sections. "The cardiac mechanism" is introduced with a discussion of the normal sequence of excitation, beginning with the S-A node and the factors influencing activity of this focus, and proceeding to various other foci

in order of complexity rather than anatomy. The effects of digitalis, quinidine, atropine, and other drugs on this mechanism, both from a diagnostic and therapeutic point of view, are discussed throughout.

The second addition to the original edition involves the introduction of the concept of the spatial vectorcardiogram. The underlying thesis of the presentation is not altered by this addition since, as the author puts it, "many problems remain to be worked out, . . . and many technical procedures require simplification and standardization." No effort is made to present the electrical activity of the heart in terms of loops and axes. As before, the instantaneous activity is discussed with respect to the classic planes described by the combined bipolar and unipolar leads and related to the various dimensions of the heart as a summation of dipoles in space.

The reader is introduced to the vectorcardiogram not as a new concept but as a new technique with which he should be familiar and to which he may further apply himself if he so desires. Since notation of the vector as the summation and resultant of activity of the entire heart in a plane was previously incorporated into the original presentation, this addition required no radical revision of the original text. The author, once more, revealed his capacity for restraint, maintaining clarity and precision without critically sacrificing completeness in his total coverage of the field. The book is recommended to the student not only as an introduction but as a useful reference upon which he can rely throughout his studies of electrocardiography.

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American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

The following dates and locations of the next three annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1958 — Philadelphia, Pennsylvania — May 18–23
1959 — Chicago, Illinois — May 24–29
1960 — Los Angeles, California — May 15–20

EMPHYSEMA CONFERENCE

A Conference on Research in Emphysema is being planned for Aspen, Colorado, for June 13–15. It will be under the joint auspices of the University of Colorado School of Medicine, the Colorado Foundation for Research in Tuberculosis, the National Tuberculosis Association, the American Trudeau Society, the Colorado Tuberculosis Association and certain local tuberculosis associations in the state, and the Colorado Trudeau Society.

Attendance will be limited to invited guests, who will also be the participants. Dr. Giles Filley of the University is chairman of the committee planning the agenda and Edward Walton, executive director of the Colorado Tuberculosis Association, is in charge of administrative details.

NAPT COMMONWEALTH CHEST CONFERENCE

The National Association for the Prevention of Tuberculosis will hold its annual chest conference in London, England, from July 1 to July 4, 1958. The scope of the Conference will be much wider than in the past as the activities of NAPT have been extended to cover diseases of the chest and heart.

Full particulars of the Conference may be obtained from the Secretary-General, NAPT, Tavistock House North, Tavistock Square, London, W. C. 1.

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1958 ANNUAL MEETING

PHILADELPHIA, PENNSYLVANIA, MAY 18–23

The 1958 Annual Meetings of the National Tuberculosis Association, the American Trudeau Society, and the National Conference of Tuberculosis Workers will be held at Convention Hall, Philadelphia, Pa., May 18 to 23.

The ATS committee meetings will be held Friday, Saturday, and Sunday, May 16, 17, and 18.

Registration will begin Saturday afternoon, May 17, and continue through Sunday evening at the Bellevue-Stratford Hotel. Beginning Monday, May 19, registration, the exhibits program, and regular daytime sessions will be held at Convention Hall. Evening sessions through the week will be held at the Bellevue-Stratford.

Medical, public health, and nursing sessions will begin on Monday, May 19. General sessions will include an opening keynote program Monday A.M., the awards session Wednesday evening, and a session Thursday morning, May 22.

Blocks of rooms have been reserved in the following hotels.

Bellevue-Stratford. Rates: single, $9–11; double, $15; twins, $12—17.
Adelphia. Rates: single, $7—10; double, $12—14; twins, $13—16.
John Bartram. Rates: single, $7; double, $9.50—10.50; twins, $10.50—12.50.
Sheraton. Rates: single, $9.85—12; twins, $14—17.
Sylvania. Rates: single, $7—7.50; twins, $11.50—13.50.
Warwick. Rates: single, $11; double, $13—16; twins, $14—19.

Special bus service will be provided between these hotels and Convention Hall.

Applications for rooms should be made directly to the hotel of choice. *Requests must include mention of NTA Annual Meeting and give a definite date and hour of arrival as well as a date of departure. Names and addresses of all persons who will occupy the rooms requested must be included.* In the event the hotel is unable to provide the desired space, requests will be referred to the Philadelphia Convention Bureau for clearing with another one of the cooperating hotels. Confirmation will come direct from hotel accepting reservation.

ANNOUNCEMENT

Now—a more palatable PAS

Despite the indispensability of para-aminosalicylic acid in tuberculosis therapy, the unfortunate drawbacks of gastric intolerance, allergic reactions, unpleasant taste, and relative instability are frequently encountered.

BENZAPAS, the calcium salt of the benzoyl derivative of PAS, has now been synthesized and demonstrated to be stable, nontoxic, equally effective, and significantly more palatable. BENZAPAS is especially useful for those patients who need PAS and yet cannot tolerate it.

In a recent paper,[1] it has been reported that BENZAPAS has better patient acceptability than PAS. Some patients who cannot tolerate sodium PAS are able to continue therapy when given Calcium Benzoyl PAS.

BENZAPAS can also be used to advantage where patients exhibit allergic reactions to PAS. In a limited series, two patients who repeatedly reacted with fever and rash to sodium PAS were able to continue treatment without difficulty when placed on BENZAPAS. Of particular importance was the observation that in no instance in this study did resistant strains emerge.

Whenever you are confronted with a patient who cannot tolerate PAS or who wants a more palatable form, you should find it of advantage to use BENZAPAS.

BENZAPAS is available as a powder in 4 gram packets, in one pound and bulk containers, and 0.5 gram tablets. The usual dose is 4 grams t.i.d. We will be pleased to send you additional information concerning this new chemotherapeutic with better patient acceptability.

[1]Dr. Samuel Phillips: *Calcium Benzoyl PAS*, Paper presented at 15th VA-Army-Navy Conference on Chemotherapy of Tuberculosis, St. Louis, Missouri, February 6-9, 1956.

G. R. Underwood, M.D.
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TRUDEAU SCHOOL OF TUBERCULOSIS

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Three Weeks, Course Beginning
JUNE 2nd

Schedule of Instruction includes the history, etiology and epidemiology; pathology, bacteriology, physiology and biochemistry; clinics and bedside teaching, including surgical and special treatments of tuberculosis; fungus infections; pneumoconioses; pulmonary tumors, and differential diagnosis of other lung disorders.

Clinical Facilities Available: Ray Brook State Tuberculosis Hospital, Will Rogers Memorial, Sanatorium Gabriels, V. A. Hospital at Sunmount, N. Y., and the General Hospital of Saranac Lake (chiefly surgical).

Research Facilities Available: Saranac Laboratory; Trudeau Laboratory; Ray Brook State Tuberculosis Hospital Laboratory and the X-Ray Laboratories of the Ray Brook State Tuberculosis Hospital and the V. A. Hospital at Sunmount.

Lectures, Demonstrations and Conferences conducted by the staffs and other experienced clinicians. By invitation, recognized authorities will give lectures on specific subjects.

Students Participate Actively in the study and discussion of cases.

Fee for Three Weeks' Course is $100.00. The class is limited, therefore early application is advised. A few scholarships are available.

Certificate: A certificate of attendance is awarded to all students who complete the course.

Note: The Trudeau School is supported by grant from the Lillia Babbitt Hyde Foundation, New York, N. Y.

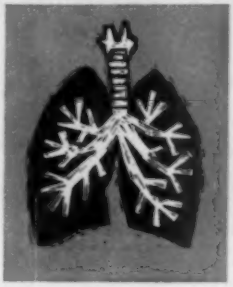
For Schedule and other information write to

Secretary, Trudeau School of Tuberculosis, Box 500,

Saranac Lake, N. Y.

ASTHMA

and allergic disorders



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		Excellent or good	Fair	Poor
Bronchial asthma 52	2-16	43	5	4
Perennial rhinitis 4	2-6	4		
Infective asthma 3	4-16	3		
Allergic asthma 2	2-10	2		
Drug allergy 1	2-16	1		

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- *and on a lower daily dosage range*

Scored tablets of 1 mg., bottles of 50 and 500. Scored tablets of 4 mg., bottles of 30 and 100.

1. Reports to the Squibb Institute of Medical Research, 1957. 2. Sherwood, H., and Cooke, R. A.: J. Allergy 28:97, 1957. 3. Hellman, L., et al.: American Rheumatism Association. Interim session, Nov. 23, 1956. 4. Berntsen, C. A., Jr., et al.: New York Rheumatism Association. Annual meeting, April 9, 1957, New York. 5. Freyberg, R. H., et al.: International Congress on Rheumatic Diseases, June, 1957, Toronto.

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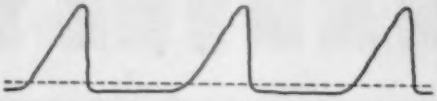

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NATIONAL TUBERCULOSIS ASSOCIATION 1904–1954

A Study of the Voluntary Health Movement in the United States

by Richard Harrison Shyrock, Ph.D

W. H. Welch Professor of the History of Medicine, and Director of the Institute of the History of Medicine, The Johns Hopkins University.

342 pages, illustrated $3.50

This book would be a valuable addition to any medical library. It should be on the reference list of every medical school; and on the desk of all private physicians interested in the problems of tuberculosis.

Not only does it supply a historical background for the antituberculosis movement in the United States, but it affords enlightening orientation to the entire voluntary health movement with the National Tuberculosis Association as the principal illustration.

The three overlapping areas of the Association's activities—medical science and practice, public health, and social welfare—are presented here by an able medical historian who knows how to give facts in a clear and interesting way.

Among the chapters are: Medical Background; Social Background; Tuberculosis in the United States; 1904; Founding of the National Tuberculosis Association; Arousing the Public, 1904–1908; Growth of the Tuberculosis Movement, 1904–1920; Administrative Developments and Reorganization; Problems and Policies, 1908–1922; Widening Contacts and Activities, 1917–1922; Foreign Contacts, War, and the Emergence of a New Era; The Research Program, 1940–1954; Chemotherapy and the Climax of Fifty Years of Service.

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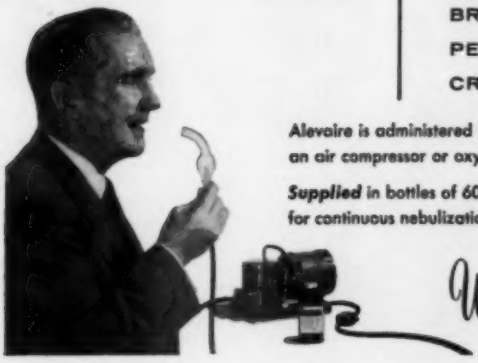

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gastrointestinal infections
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- when the patient is comatose or in shock
- postoperatively

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2. high cerebrospinal levels
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Vocational Rehabilitation of the Tuberculous

by Sol L. Warren, Ph.D.

135 pages, paper cover $2.00

This volume should be in every medical library and medical school as a reference text. Every physician working with tuberculosis patients will find this book exceedingly valuable.

Essentially, Dr. Warren's book describes the study he conducted to determine whether any differences could be found between a group of ex-tuberculosis sanatorium patients who, following discharge, participated in a state agency vocational rehabilitation program, and a group which did not participate.

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